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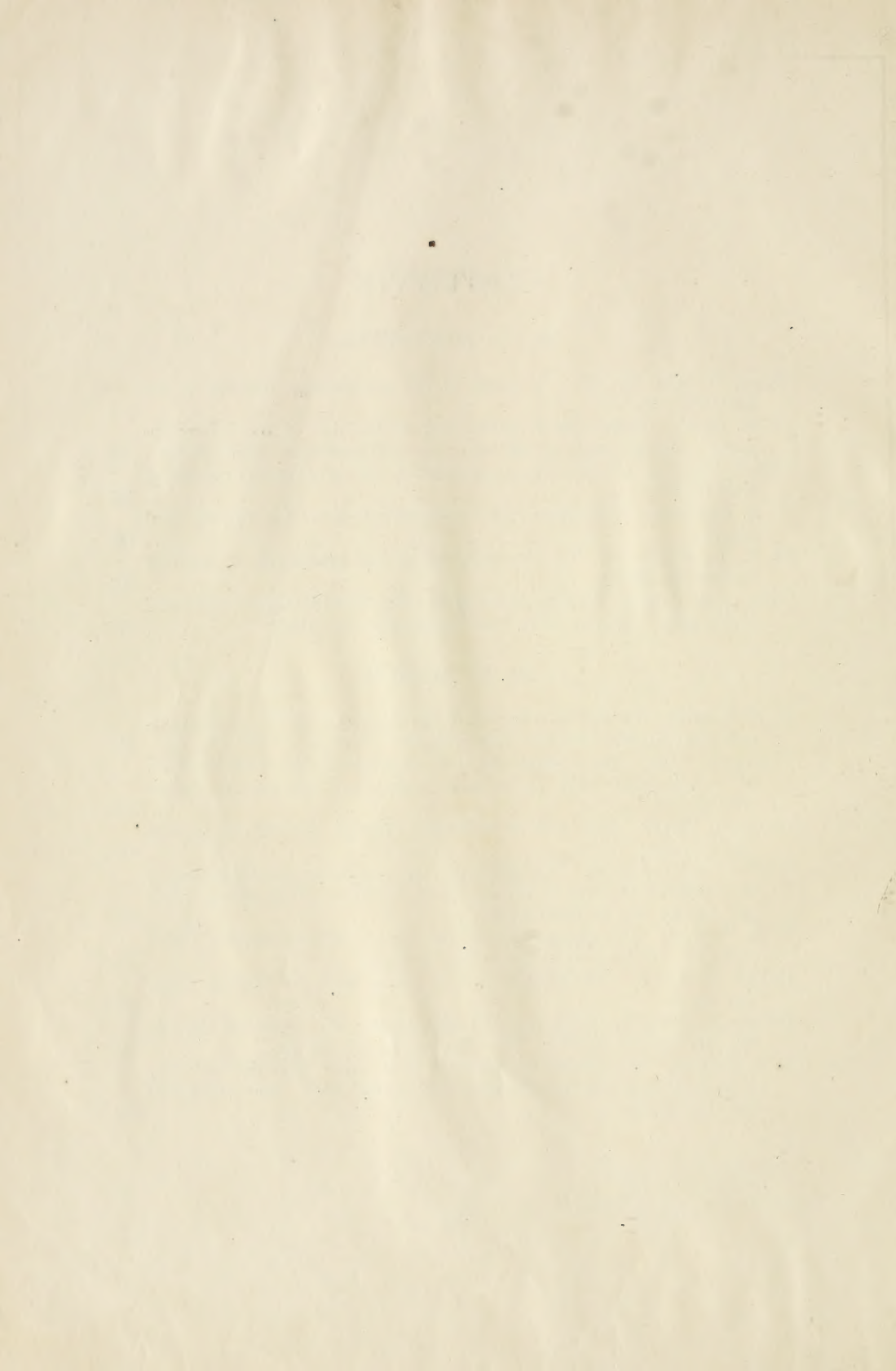
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A REVIEW OF THE CHROMOSOME NUMBERS IN THE METAZOA

PART I

ETHEL BROWNE HARVEY

Cornell University Medical School

The following tabulation comprises the chromosome work that has been done since the pioneer researches of Van Beneden in 1883 until the end of 1915. The list is as complete as possible, and recourse has been had in all cases to the original publications. All the authorities have been given for each species, even when the accounts are conflicting, and these are given in the order of priority. All the species are classified and the various phyla, genera, families and species are arranged in alphabetical order. The present paper includes the Annelida, Arthropoda and Coelenterata, and will be followed by a second paper including a tabulation of the other invertebrates (excluding Protozoa) and the vertebrates together with some general remarks. Some of the most important abbreviations are:

chrom = chromosome
cl = cleavage
div = division
el = elements
fert = fertilized
mat = maturation
oog = oögonia

pa = parthenogenetic
p.b. = polar body
prim = primordial
pron = pronucleus
som = somatic
spc = spermatocyte
spg = spermatogonia

"X to pole" means that X passes undivided to one pole.

"XY to poles" means that X and Y pass to opposite poles.

"From correction in" means that the tabulation as given is a correction of an earlier account.

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
A. INVERTEBRATA							
I. ANNELIDA							
a. ARCHANNELIDA							
<i>Dinophilus gyrocolliatus</i> ...	20 cl ♂ 20 cl ♀	20 ♂ egg (= 10 double) 20 ♀ egg (= 10 double)	10 ♂ egg (double)	10 ♂ egg (double)		Shearer, '11 Shearer, '12	Jour. Marine Biol. As- soc. 9, p. 156 Q. J. M. S., 57, p. 329
<i>Histiobdella homari</i> <i>Protodrilus purpureus</i>	8 ♀ 4 ♀		4 ♀	4 ♀		Shearer, '10 Pierantoni, '08	Q. J. M. S. 55, p. 287 Flora u. Fauna Golfes Neapel, 31, p. 1
b. CHAETOPODA							
1. <i>Oligochaeta</i>							
<i>Allobophora foetida</i>	22 oeg	11 ♀	11 ♀	11 ♀		Foot, '98 Foot and Strobell, '03 Foot and Strobell, '13 Vejdovský, '07	Jour. Morph., 14, p. 481 Amer. Jour. Anat., 4, p. 199 Arch. Zellf., 5, p. 149 Kgl. böhm. Ges. Wiss. Prag, '07, p. 1
<i>Enechytraeus adriaticus</i> ...	24 oeg					Vejdovský, '07	Kgl. böhm. Ges. Wiss. Prag, '07, p. 1
<i>Enechytraeus humiculator</i> .	32 spg 32 oeg	16 ♀				Vejdovský, '07	Kgl. böhm. Ges. Wiss. Prag, '07, p. 1
<i>Fridericia hegemon</i>	64 spg 64 oeg	32 ♀				Vejdovský, '07	Kgl. böhm. Ges. Wiss. Prag, '07, p. 1
<i>Ilyodrilus cocineus</i>		16 ♀				Vejdovský, '07	Kgl. böhm. Ges. Wiss. Prag, '07, p. 1
<i>Lumbricus agricola</i>		16 ♂ (= 64 el)	16 ♂ (= 32 el)	16 ♂		Vejdovský and Mrázek, '03 Burnion and Po- poff, '05	Arch. mikr. Anat., 62, p. 431 Arch. Zool. exper. et gen., Ser. IV, vol. 2, p. 339
<i>Lumbricus terrestris</i>	32 spg	16 ♂	16 ♂	16 ♂		Calkins, '95	Jour. Morph., 11, p. 271
<i>Lumbricus</i> (sp. not given)	32 spg	16 ♂	16 ♂			Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Mesenchytraeus flavus</i> ...		16 ♀				Vejdovský, '07	Kgl. böhm. Ges. Wiss. Prag, '07, p. 1

2. <i>Polyclada</i>					
a. Archiclaetopoda					
<i>Mesenchytraeus setosus</i> ...	32 oog	16 ♀			Vejdovský, '07 Kgl. böhm. Ges. Wiss. Prag, '07, p. 1
<i>Rhynchelmis limosella</i> ...	64 cl	32 ♀	32 ♀		Vejdovský and Mrázek, '03 Kgl. böhm. Ges. Wiss. Prag, '07, p. 1
<i>Tubifex rivulorum</i>	ca 100 cl	ca. 110 ♀			Gathy, '00 La Cellule, 17, p. 7
<i>Saccocirrus major</i>	8 spg	4 ♂ 4 ♀	4 ♂ 4 ♀	4 ♀	Hempelmann, '13 Zoologica 26, p. 249 (= Festschrift für Chun)
<i>Saccocirrus major</i>	18 spg 18 oog 18 cl	9 ♂ 9 ♀	9 ♂	9 ♂	Von Baehr, '13 Zool. Anz., 43, p. 10
<i>Saccocirrus papilloereus</i> .	6 cl				Pierantoni, '06 Mitt. Zool. St. Neapel, 18, p. 46
<i>Saccocirrus papilloereus</i> .	8 spg	4 ♂ 4 ♀	4 ♂ 4 ♀	4 ♀	Hempelmann, '13 Zoologica 26, p. 249 (= Festschrift für Chun)
b. Errantia					
<i>Nereis limbata</i>	20-30 cl	14 ♀			Bonnevie '07 Bonnevie, '08 Biol. Bull., 13, p. 57 Arch. Zellf., 2, p. 201
<i>Platynereis megalops</i>		14 ♀		14 ♀	Just, '15 Jour. Morph., 26, p. 217
<i>Ophryotrocha puerilis</i>	4 spg 4 oog 4 som 4 cl 8 in some blastulae	2 ♀	2 ♀	2 ♀	Korschelt, '95 Verh. deutsch. Zool. Gesell., 5, p. 96 Zeits. wiss. Zool., 60, p. 543
<i>Ophryotrocha puerilis</i>	8 spg 8 oog 8 som	4 ♂ 4 ♀	4 ♂		Schreiners, '06 Anat. Anz., 29, p. 465
<i>Ophryotrocha puerilis</i>	8 spg 8 som	4 ♂	4 ♂		La Cellule, 23, p. 433
<i>Ophryotrocha puerilis</i>	4 (pairs) spg 4 (pairs) som	4 (pairs) ♂	2 (pairs) ♂	2 (pairs) ♂	Zool. Anz., 36, p. 209 Arch. Zool. exper. et gen., Ser. V. vol. 9, p. 1

I. ANNELIDA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Tomopteris elegans</i>	10 oog	5 ♀				Senna, '11	Arch. ital. Anat. Emb., 9, p. 299
<i>Tomopteris onisciformis</i> ..		4 ♀				W. Wallace, '04	Brit. Assoc. Adv. Sci., 73, p. 282
<i>Tomopteris onisciformis</i> ..	18 spg	9 ♂				Schreiners, '06	Arch. Biol., 22, p. 1
c. Sedentaria							
<i>Amphitrite</i>	22 cl 11 pa cl	11 ♀	11 ♀	11 ♀	2 p.b.s. and 11 pa cl. Sometimes no p.b. and 22 pa cl	J. W. Scott, '06	Jour. Exp. Zool., 3, p. 49
<i>Aricia</i>	18 cl 9 pa cl	9 ♀	9 ♀	9 ♀	2 p.b.s. and 9 pa cl. Sometimes 1 p.b. and 18 pa cl	Kostanecki, '09	Bull. Inter. Acad. Sci. Chacovic, '09, p. 238
<i>Chaetopterus pergamen- taceus</i>	18 cl	9 ♀	9 ♀	9 ♀		Mead, '98	Jour. Morph., 14, p. 181
<i>Chaetopterus pergamen- taceus</i>		9 ♀	9 ♀			Lillie, '06	Jour. Exp. Zool., 3, p. 153
<i>Lanice conchylega</i>	6 (pairs) som	6 (pairs) ♂	3 (pairs) ♂	3 ♂		Dehorne, '11	Arch. Zool. exper. et. gen., Ser. V, vol. 9, p. 1
<i>Subellaria spinulosa</i>	8 (pairs) spg 8 (pairs) som	8 (pairs) ♂ 8 (pairs) ♀	4 (pairs) ♂ 4 (pairs) ♀	4 (pairs) ♀		Dehorne, '10 Dehorne, '11	C. R. Acad. Sci. Paris, 150, pp. 1195 and 1625 Arch. Zool. exper. et gen., Ser. V, vol. 9, p. 1
<i>Serpula crater</i>		ca 14 ♀		ca 7 ♂ pron ca 7 ♀ pron		Soutier, '06	Arch. Zool. exper. et gen., Ser. IV, vol. 5, p. 403
d. Appendix-Myzostomidae							
<i>Myzostoma glabrum</i>	24 cl	12 ♀	12 ♀	12 ♂ pron 12 ♀ pron		Wheeler, '97	Arch. Biol., 15, p. 1

c. ГЕРИУРЕА
1. *Armata*

<i>Thalassema mellita</i>	24 cl 12 pa cl	12 ♀ 12 ♀	12 ♀	Griffen, '99 Lefevre, '06 Lefevre, '07	Jour. Morph., 15, p. 583 Science, 23, p. 522 Jour. Exp. Zool., 4, p. 91
<i>Thalassema mellita</i>		12+ ♀	16-17 ♀	Bonnevie, '08	Arch. Zellf., 2, p. 201

2. *Inermia*

<i>Phascolosoma gouldii</i>	20 cl (= 10 bi-valent in vulgare)	10 ♀	10 ♀	Gerould, '04	Arch. Zool. exper. et gen. Ser. IV, vol. 2, p. XVII
<i>Phascolosoma vulgare</i>				Gerould, '06	Zool. Jahrb., 23, p. 77
<i>Sipunculus nudus</i>		10 ♀		Gerould, '06	Zool. Jahrb., 23, p. 77

d. HIRUDINEA

<i>Clepsine complanata</i>	16 oog	20 ♀		Gathy, '00	La Cellule, 17, p. 7
<i>Nephele vulgaris</i> = <i>Herpobdella atomaria</i>		8 ♀		Jørgensen, '08	Arch. Zellf., 2, p. 279
<i>Piscicola</i>		16 ♀		Jørgensen, '13	Arch. Zellf., 10, p. 127

II. ARTHROPODA

a. ARACHNIDA

1. *Acarida*

<i>Ixodes reduvius</i>	ca 28 spg	14 ♂		Nordenskiöld, '09	Zool. Anz., 34, p. 511
<i>Pediculus graminum</i>	4 cl (some-times fused)		2 ♂ pron 2 ♀ pron	Reuter, '09	Acta Soc. Sc. Fen., 37, no. 7, p. 1

2. *Araeidea*

<i>Agalena naevia</i>	52? spg		25, 27? ♂	L. B. Wallace, '00 L. B. Wallace, '05 L. B. Wallace, '09	Anat. Anz., 18, p. 327 Biol. Bull., 8, p. 169 Biol. Bull., 17, p. 120
<i>Agalena naevia</i>		ca 15 ♂	17+ ♂	Painter, '14	Zool. Jahrb., 38, p. 509
<i>Amaurobius sylvestris</i>				Painter, '14	Zool. Jahrb., 38, p. 509
<i>Anypaena saltibunda</i>		10 ♂		Painter, '14	Zool. Jahrb., 38, p. 509

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST CYTE	2ND CYTE	TID	REMARKS	OBSERVER	REFERENCE
<i>Callipes hubbilla</i>		11♂			X to pole in 1st	Painter, '14	Zool. Jahrb., 38, p. 509
<i>Clubiona</i>		22-26♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Dugesia lentzi</i>		22♂			X to pole in 1st	Painter, '14	Zool. Jahrb., 38, p. 509
<i>Dolomedes fontanus</i>		13♂			Double X to pole in 1st	Painter, '14	Zool. Jahrb., 38, p. 509
<i>Epeira scolopetaria</i>	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Berry, '06	Biol. Bull., 11, p. 193
<i>Lycosa communis</i>		11♂			X to pole in 1st	Painter, '14	Zool. Jahrb., 38, p. 509
<i>Lycosa inops</i>	28 spg	13♂	13♂ (12-15)		Pair small chroms. in spg usually absent in spc. Equal XY	Montgomery, '05	Proc. Acad. Nat. Sci. Phila., 57, p. 162
<i>Maevia vittata</i>	28+ spg 20+ oog	15♂			X to pole in 1st	Painter, '14	Zool. Jahrb., 38, p. 509
<i>Oxyopes salticus</i>		11♂			X to pole in 1st. Supernumeraries	Painter, '14	Zool. Jahrb., 38, p. 509
<i>Teguarina atrica</i>		18-24♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Tegonaria</i> sp?.....		6-12♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Theridium topidariorum</i>	24 cl	12♀	12♀	12♀		Montgomery, '07	Zool. Jahrb., 25, p. 237
<i>Xysticus triguttatus</i>		11♂			X to pole in 1st	Painter, '14	Zool. Jahrb., 38, p. 509

3. <i>Scorpionidae</i>						
<i>Butkus eupeus</i>	ca 22 spg					Sokolow, '13
<i>Eusecorpius carpathicus</i>	70-84 spg	28, 40♂ (probably 32)	28-40♂			Sokolow, '13
<i>Scorpio occitanus</i> (= <i>Butkus</i>).....		22-28♂				Carnoy, '85
4. <i>Appendix—Tardigrada</i>						
<i>Macrobiotus laevis</i>	10 cl	5♀	5♀	5♀		Von Weneck, '14
						Zool. Jahrb. Abt. f. Anat., 37, p. 463

b. CRUSTACEA
1. *Entomostraca*
a. Cirripedia

Lepas anatifera.....	16 ♀ (double) oog 32 som	16 ♀ (= 64 el)	16 ♀ (= 32 el)	4-12 ♀ (mostly 4 or 5)	Groom, '94	Phil. Trans. Roy. Soc., 185 B, p. 119
b. Copepoda						
Anomaloecra patersonii.....	16 (double) oog 32 som	16 ♀ (= 64 el)	16 ♀ (= 32 el)	16 ♀	Vom Rath, '95	Arch. mikr. Anat., 46, p. 168
Calanus gracilis.....		16 ♀			Konrhauser, '15	Arch. Zellf., 13, p. 399
Canthocamptus staphy- linus.....	24 oog ca 12 (double) cl	12 ♀ (= 48 el)	6 pairs ♀ (= 24 el)	6 ♀ (= 12 el)	Häcker, '92 Häcker, '95	Zool. Jahrb., 5, p. 211 Arch. mikr. Anat., 45, p. 200
Canthocamptus staphy- linus.....		12 ♀	12 ♀	12 ♀	Matschek, '09 Matschek, '10	Zool. Anz., 34, p. 42 Arch. Zellf., 5, p. 36
Canthocamptus staphy- linus.....		12 ♀ (double)			Krüger, '11	Arch. Zellf., 6, p. 165
Canthocamptus trispin- osus.....		11 ♀ (double)			Krüger, '11	Arch. Zellf., 6, p. 165
Cyclops affinis.....		7 ♀			Matschek, '10	Arch. Zellf., 5, p. 36
Cyclops albidus.....	14 som	7 ♀	7 ♀		Braun, '09	Arch. Zellf., 3 p. 449
Cyclops albidus.....		7 ♀			Matschek, '09 Matschek, '10	Zool. Anz., 34, p. 42 Arch. Zellf., 5, p. 36
Cyclops albidus.....	14				Chambers, '12	Univ. Toronto Studies, 14, p. 1
Cyclops bicuspidatus.....	18 som	9 ♀			Braun, '09	Arch. Zellf., 3, p. 449
Cyclops bicuspidatus.....		9 ♀			Matschek, '09 Matschek, '10	Zool. Anz., 34, p. 42 Arch. Zellf., 5, p. 36
Cyclops bicuspidatus.....	18				Chambers, '12	Univ. Toronto Studies 14, p. 1.
Cyclops bicuspidatus var. odessana.....	18 som	9 ♀			Braun, '09	Arch. Zellf., 3, p. 449
Cyclops bicuspidatus var. odessana.....		9 ♀			Matschek, '10	Arch. Zellf., 5, p. 36

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Cyclops brevicornis</i> see <i>Cyclops viridis</i>							
<i>Cyclops diaphanus</i>	12 som	6 ♀			Ditetrads	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops distinctus</i> see <i>fuscus</i>							
<i>Cyclops dybowskii</i>	18 som	9 ♀			Ditetrads	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops dybowskii</i>		9 ♀			Ditetrads	Matscheck, '09 Matscheck, '10	Zool. Anz., 34, p. 42 Arch. Zellf., 5, p. 36
<i>Cyclops fuscus</i>	14 som	7 ♀			Ditetrads	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops fuscus</i>	7 oog	7 ♀			Ditetrads	Matscheck, '10	Arch. Zellf., 5, p. 36
<i>Cyclops fuscus</i>	14					Chambers, '12	Univ. Toronto Stud- ies 14, p. 1
<i>Cyclops fuscus</i> var. <i>dis-</i> <i>tinctus</i> (possibly hy- brid <i>fuscus</i> x <i>albidus</i>)...	11 som	6 ♀			Ditetrads	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops fuscus</i> var. <i>dis-</i> <i>tinctus</i>		6, 7 ♀			5 or 6 ditetrads + 1 heterochromosome	Matscheck, '10	Arch. Zellf., 5, p. 36
<i>Cyclops fuscus</i> var. <i>dis-</i> <i>tinctus</i>	11 prim. germ cells 11 cl					Amma, '11	Arch. Zellf., 6, p. 497
<i>Cyclops gracilis</i>	6 som	3 ♀			Ditetrads	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops gracilis</i>	6 cl	3 ♀	3 ♀	3♂ pron 3 ♀	Ditetrads	Matscheck, '09 Matscheck, '10	Zool. Anz., 34, p. 42 Arch. Zellf., 5, p. 36
<i>Cyclops insignis</i>	22 som	11 ♀			Ditetrads	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops insignis</i>		11 ♀			Ditetrads	Matscheck, '10	Arch. Zellf., 5, p. 36
<i>Cyclops insignis</i>	22 prim. germ cells					Amma, '11	Arch. Zellf., 6, p. 497
<i>Cyclops leuckarti</i>	14 som	7 ♀			Ditetrads	Braun, '09	Arch. Zellf., 3, p. 449

<i>Cyclops leuckarti</i>		7 ♀			Ditetrads	Matschek, '10	Arch. Zellf., 5, p. 36
<i>Cyclops modestus</i>	8					Chambers, '12	Univ. Toronto Studies 14, p. 1
<i>Cyclops phaleratus</i>	13 som	7 ♀			6 ditetrads + 1 heterochromosome	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops phaleratus</i>	7 Keimpolster	7 ♀	6, 7 ♀	6, 7 ♀	6 ditetrads + 1 heterochrom. Latter to pole in 1st	Matschek, '09 Matschek, '10	Zool. Anz., 34, p. 42 Arch. Zellf., 5, p. 36
<i>Cyclops prasinus</i>	11 som	6 ♀			5 ditetrads + 1 heterochromosome	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops prasinus</i>		6 ♀			5 ditetrads + 1 heterochromosome	Matschek, '10	Arch. Zellf., 5, p. 36
<i>Cyclops serrulatus</i>	14 som	7 ♀		6, 7 ♀	6 ditetrads + 1 metachrom. Latter to either pole in 2nd	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops serrulatus</i>		8 ♀			6 ditetrads + 2 heterochroms	Matschek, '10	Arch. Zellf., 5, p. 36
<i>Cyclops signatus</i>	8 oog 8 cl (double)	8 ♀ (= 16 cl)	4 ♀ (= 8 cl)	4 ♂ 4 ♀		Häcker, '90 Häcker, '92	Zool. Anz., 13, p. 551 Zool. Jahrb., 5, p. 211
<i>Cyclops strenuus</i>	8 oog 8 cl (double)	8 ♀ (= 16 cl)	4 ♀ (= 8 cl)	4 ♂ 4 ♀		Häcker, '90 Häcker, '92 Häcker, '93 Häcker, '94	Zool. Anz., 13, p. 551 Zool. Jahrb., 5, p. 211 Arch. mikr. Anat., 41, p. 452 Arch. mikr. Anat., 43, p. 759
<i>Cyclops strenuus</i>	22-24 cl	11-12 ♀		11-12 ♂ pron 11-12 ♀ pron		Rückert, '94 Rückert, '95	Anat. Hefte, 4, p. 261 Arch. mikr. Anat., 45 p. 339
<i>Cyclops strenuus</i>	ca 20 spg	11 ♀				Lerat, '05	La Cellule, 22, p. 161
<i>Cyclops strenuus</i>	22 som	11 ♀ 14 ♀			Ditetrads. May be 2 varieties	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops strenuus</i>		11 ♀			Ditetrads	Matschek, '10	Arch. Zellf., 5, p. 36
<i>Cyclops strenuus</i>	22 cl					Amma, '11	Arch. Zellf., 6, p. 497
<i>Cyclops vernalis</i>	10 som	5 ♀			Ditetrads	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops vernalis</i>		5, 6 ♀			5 ditetrads or same + 1 heterochrom	Matschek, '09 Matschek, '10	Zool. Anz., 34, p. 42 Arch. Zellf., 5, p. 36

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Cyclops viridis</i> = <i>brevicornis</i>	12 cl (double) = 24	12 ♀ (= 24)	6 pairs ♀ (= 12)	6♂ pron (= 12) 6 ♀		Häcker, '95 Häcker '97 Häcker, '03 Häcker, '04	Arch. mikr. Anat., 46, p. 579 Biol. Centralb., 17, p. 721 Jen. Zeits., 30, p. 297 Zool. Jahrb., 7, suppl., p. 161
<i>Cyclops viridis</i>	12 cl 14 cl				2 varieties	Schiller, '08	Zool. Anz., 32, p. 616
<i>Cyclops viridis</i>	12 som	6 ♀			Ditetradis	Braun, '09	Arch. Zellf., 3, p. 449
<i>Cyclops viridis</i>	12 spg 12 oog					Krimmel, '10	Zool. Anz., 35, p. 778
<i>Cyclops viridis</i>		6 ♀			Ditetradis	Matscheck, '10	Arch. Zellf., 5, p. 36
<i>Cyclops viridis</i>	12 cl	6 ♀			Biserial	Amma, '11	Arch. Zellf., 6, p. 497
<i>Cyclops viridis</i>	12					Chamber-, '12	Univ. Toronto Studies 14, p. 1
<i>Cyclops viridis</i> var. <i>americanus</i>	10 spg 10 oog 10 som	5♂ 5 ♀	5♂ 5 ♀	5♂ 5 ♀	Biserial	Chambers, '12	Univ. Toronto Studies, 14, p. 1
<i>Cyclops viridis</i> var. <i>brevispinosus</i>	4 oog	2 ♀	2 ♀		Biserial	Chambers, '12	Univ. Toronto Studies, 14, p. 1
<i>Cyclops viridis</i> var. <i>parcus</i>	6 oog	3 ♀	3 ♀	3♂ 3 ♀	Biserial	Chambers, '12	Univ. Toronto Studies, 14, p. 1
<i>Diaptomus castor</i>		15 ♀	17 ♀		1 heterochrom = 3 elements, are sep- arate in 2nd and divide in both di- visions	Häcker and Mat- scheck, '08 Matscheck, '09 Matscheck, '10	Verh. deutsch. Zool. Ges., 18, p. 110 Zool. Anz., 34, p. 42 Arch. Zellf., 5, p. 36

Diaptomus castor.....	34 cl	14 ♀		17♂ pron 17♀ pron	Amma, '11	Arch. Zellf., 6, p. 497
Diaptomus coereuleus.....					Häcker and Matscheck, '08	Verh. deutsch. Zool. Ges., 18, p. 110
Diaptomus coereuleus.....	28 spg (14-28) 28 oög 28 som (14-28) 28 cl				Matscheck, '09	Zool. Anz., 34, p. 42
Diaptomus coereuleus.....					Krimmel, '10	Zool. Anz., 35, p. 778
Diaptomus denticornis.....	32 cl			14♂ pron 14♀ pron	Amma, '11	Arch. Zellf., 6, p. 497
Diaptomus denticornis.....		17 ♀			Häcker, '03	Jen. Zeits., 30, p. 297
Diaptomus gracilis.....					Matscheck, '09	Zool. Anz., 34, p. 42
Diaptomus gracilis.....	32 cl	16 ♀			Matscheck, '10	Arch. Zellf., 5, p. 36
Diaptomus gracilis.....		17 ♀			Rückert, '94	Anat. Hefte 4, p. 261
Diaptomus laciniatus.....		16 ♀			Häcker and Matscheck, '08	Verh. deutsch. Zool. Ges., 18, p. 110
Diaptomus salinus.....		17 ♀			Matscheck, '09	Zool. Anz., 34, p. 42
Diaptomus sp? (Japanese).....	8 spg 8 oög 8 cl	8♂ 8♀	8♂ 8♀	4♂ 4♀	Matscheck, '10	Arch. Zellf., 5, p. 36
Euchaeta marina.....		12 ♀ (= 48 el)	12 ♀ (= 24 el)	12 ♀	Ishikawa, '91	Coll. Sci. Imp. Univ. Japan, 5, p. 1
Euchaeta marina.....		17 ♀			Vom Rath, '95	Arch. mikr. Anat., 46, p. 168
Hersilia apodiformis.....	24 spg 24 oög 24 cl	12♂ 12♀	12♂		Kornhauser, '15	Arch. Zellf., 13, p. 399
Heterocope robusta.....		16 ♀			Kornhauser '15	Arch. Zellf., 13, p. 399
Heterocope saliens.....		16 ♀			Rückert, '94	Anat. Hefte, 4, p. 261
Heterocope weismanni.....		16 ♀			Matscheck, '09	Zool. Anz., 34, p. 42
					Matscheck, '10	Arch. Zellf., 5, p. 36
					Häcker and Matscheck, '08	Verh. deutsch. Zool. Ges., 18, p. 110
					Matscheck, '09	Zool. Anz., 34, p. 42
					Matscheck, '10	Arch. Zellf., 5, p. 36

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Laemargus muricatus</i>	16 spg 16' oog	8♂	8♂	8♂		McClendon, '06	Biol. Bull., 12, p. 37
<i>Lichonolagus forficula</i>		10♀				Kornhauser, '15	Arch. Zellf., 13, p. 399
<i>Mytilicola intestinalis</i>	8 spg 8 oog	16♂	8♂	4♂		Steuer, '03	Arch. Zool. Inst. Wien, 15, p. 1
<i>Orthazoriseicola muricata</i>	16 oog	8♀				McClendon, '06	Biol. Bull., 12, p. 37
<i>Nitocera hibernica</i>		8♀ (double)				McClendon, '10	Arch. Zellf., 5, p. 229
<i>Pandarus sinuatus</i>	16 spg 16 oog	8♂ 8♀	8♂ 8♀	8♂		Krüger, '11	Arch. Zellf., 6, p. 165
<i>Paracalanus parvus</i>						McClendon, '06	Biol. Bull., 12, pp. 37 and 63
<i>Sapphirina</i> sp?.....	16 spg	6♀				McClendon, '07	Biol. Bull., 13, p. 114
						McClendon, '10	Arch. Zellf., 5, p. 229
						Moroff, '09	Arch. Zellf., 2, p. 432
						Kornhauser, '15	Arch. Zellf., 13, p. 399

c. Ostracoda							
<i>Cypris fuscata</i>	24 cl	24♀			Pa egg; 1 p.b., no reduction	Schleip '09	Arch. Zellf., 2, p. 390
<i>Cypris incongruens</i>	12 pa cl	12♀			Pa; 1 p.b.	Volterreck, '98	Zeit. wiss. Zool., 64, p. 396
<i>Cypris incongruens</i>	12 pa cl	12♀			Pa; 1 p.b., no re- duction. No X	Müller-Calé, '13	Zool. Jahrb., 36, p. 113.
<i>Cypris reptans</i>	12 pa cl	12♀			Pa; 1 p.b.	Volterreck, '98	Zeit. wiss. Zool., 64, p. 396
<i>Notodromas monacha</i> = <i>Cypris monacha</i>	13-16 oog 16 cl	8♀	8♀	8♂ 8♀		Schleip, '09	Arch. Zellf., 2, p. 390
<i>Notodromas monacha</i>	16 cl	8♂	8♂	8♂		Schmalz, '11 Schmalz, '12	Zool. Anz., 37, p. 462 Arch. Zellf., 8, p. 407

d. Phyllopoda
1. Cladocera

<i>Bythotrephes longimanus</i>	4? cl		2? ♀	Pa eggs; 1 p.b., no reduction	Weismann and Ishikawa, '89	Zool. Jahrb., 4, p. 155
<i>Daphnia pulex</i>	8 spg 8 som 8 cl	8 ♀			Kühn, '08	Arch. Zellf., 1, p. 538
<i>Daphnia pulex</i>	8-10 spg 8-10 som	4-5 ♂ ^a	4-5 ♂ ^a	Winter egg	Taylor, '14	Zool. Anz., 45, p. 21
<i>Meina paradoxa</i> } <i>Meina rectirostris</i> }		4 ♀	4 ♀		Weismann and Ishikawa, '89	Zool. Jahrb., 4, p. 155
<i>Polyphemus pediculus</i> ...	8 spg 8 som 8 cl	8 ♀			Kühn, '08	Arch. Zellf., 1, p. 538
<i>Simoccephalus vetulus</i> ...		8 ♂ ^a	ca 8 ♂ ^a	No X	Chambers, '13	Biol. Bull., 25, p. 134

2. Euphylopoda

<i>Apus</i>	1 oog			Monomeric division	Moore, '93	Q. J. M. S., 35, p. 259
<i>Artemia salina</i>		30-40 ♀		Pa; 1 p.b.	Weismann and Ishikawa, '88	Zool. Jahrb., 3, p. 575
<i>Artemia salina</i>	84 cl 168 cl	84 ♀	84 ♀	Pa; pronucleus may be fertilized by 2nd p.b. (= 168)	Brauer, '93 Brauer, '94	Zool. Anz., 16, p. 138 Arch. mikr. Anat., 43, p. 162
<i>Artemia salina</i>		84 ♀	84 ♀	Pa; 1 p.b.	Petrunkewitsch, '02	Anat. Anz., 21, p. 256
<i>Artemia salina</i>	80-90 oog (= 84?) 70-80 som	84 ♀			Fries, '09	Arch. Zellf., 4, p. 44
<i>Artemia salina</i> var. <i>bivalens</i> parthenogenetica di Capo d'Istria...	84 cl	84 ♀		Pa; 1 p.b.	Artom, '08 Artom, '11 Artom, '11	Biologica 1, pp. 5 and 435 Arch. Zellf., 7, p. 277 Biol. Centralb., 31, p. 104
<i>Artemia salina</i> var. <i>univalens</i> sessuata di Cagliari.....	42 cl	21 ♀	21 ♀	Sexual	Artom, '12 Artom, '08 Artom, '11 Artom, '11	Arch. Zellf., 9, p. 87 Biologica 1, pp. 5 and 435 Arch. Zellf., 7, p. 277 Biol. Centralb., 31, p. 104

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Branchipus</i>		10♂ (double)	10♂ (double)			Moore, '93	Q. J. M. S., 35, p. 259
<i>Branchipus grubii</i>	24 oog; 24 cl	12♀	12♀	12♀		Brauer, '92	Abh. Akad. Wiss. Ber- lin, Physik. Abt., '92, p. 1
						Brauer, '94	Arch. mikr. Anat., 43, p. 162
<i>Branchipus grubii</i>	22-24 oog 24 som	12♀		12♂ pron 12♀ pron		Fries, '09	Arch. Zellf., 4, p. 44
<i>2. Malacostraca</i>							
<i>a. Arthrostraca</i>							
<i>1. Amphipoda</i>							
<i>Talorchestia longicornis</i> ..		18♂				Nichols, '09	Jour. Morph., 20, p. 461
<i>2. Isopoda</i>							
<i>Asellus aquaticus</i>	20-30 spg	8-10♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Idotea irrorata</i>		28♂				Nichols, '09	Jour. Morph., 20, p. 461
<i>Oniscus asellus</i>		16♂				Nichols, '02	Proc. Amer. Phil. Soc., 41, p. 77
						Nichols, '09	Jour. Morph., 20, p. 461
<i>b. Decapoda</i>							
<i>1. Brachyura</i>							
<i>Carcinus menas</i>		30-40♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Menippe mercenaria</i>	51-80 spg		25-28♀			Binford, '13	Jour. Morph., 24, p. 147
<i>2. Macrura</i>							
<i>Astacus fluviatilis</i>		ca 58♂				Prowazek, '02	Zeits. wiss. Zool., 71, p. 445
<i>Cambarus immunis</i> ?.....		104♂	104♂		A clump of 8 chiroms	Fasten, '14	Jour. Morph., 25, p. 557

Cambarus virilis.....	200 spg	100♂	100♂	Fasten, '14	Jour. Morph., 25, p. 587
Crangon cataphractus.....		40-44♂		Carnoy, '85	La Cellule, 1, p. 189
Eupagurus prideauxii.....		12♀	12♀	Weismann and Ishikawa, '88	Zool. Jahrb., 3, p. 575
Euphasidae.....	33 cl			Taube, '09	Zeits. wiss. Zool., 92, p. 427
Hippa talpoides.....		60♂		Nichols, '09	Jour. Morph., 20, p. 461
Homarus.....		18♂		Labbé, '04	C. R. Acad. Sci. Paris, 138, p. 96

c. Stomatopoda					
Squilla mantis.....		20-24♂		Carnoy, '85	La Cellule, 1, p. 189

c. INSECTA					
I. Apter					
a. Collembola					
Anurida maritima.....	8 oog 87 cl			Claypole, '98	Jour. Morph., 14, p. 219
Orechesella villosa.....		6?♀		Lécaillon, '01	Arch. d'Anat. mikr., 4, p. 471
Podura aquatica.....	8 oog			Willem, '00	Mém. couronnées Acad. roy. Belgique 68, no. 3, p. 1

2. Coleoptera					
a. Buprestidae					
Euchromia gigantea.....		13♂		Nichols, '10	Biol. Bull., 19, p. 167
Spruce-borer (unidentified).....		10♂	10♂	Stevens, '06	Carnegie Inst. Pub., 36 II, p. 33
Spruce-borer (another sp).....		11♂		Stevens, '06	Carnegie Inst. Pub., 36 II, p. 33

II. ARTHROPODA—Continued

b. Carabidae

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Anomoglossus emarginatus</i>		19♂	18, 19♂	15, 19♂	X to pole in 1st	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Chlaenius aestivus</i>		17♂			XY to poles in 1st	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Chlaenius pennsylvanicus</i>		10♂			XY to poles in 1st	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Galerita bicolor</i>	30 spg				XY	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33

c. Cerambycidae

<i>Cylene robinia</i>	20 spg	10♂			XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Tetraopes tetraophthalmus</i>	20 spg	10♂			XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101

d. Chrysomelidae

<i>Adimonia tanaeti</i>		12 (at least) ♀				Henking, '92	Zeit. wiss. Zool., 54, p. 1
<i>Agelastica alni</i>	24-25 spg 24-30 cl	ca. 12♂ (= 16 -17 cl) 12 ♀	ca. 12♂ (= 16 -17 cl) 12 ♀			Henking, '92	Zeit. wiss. Zool., 54, p. 1
<i>Blepharida rhois</i>		16♂	16♂	16♂	XY to poles in 1st	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Chelymorphism argus</i>	22 spg 22 ♀ som	11♂	11♂		XY to poles in 1st XX in ♀ diploid	Stevens, '06	Carnegie Inst. Pub. 36, II, p. 33

e. Cincindelidae

<i>Cincindella prineriana</i>	20 spg	10♂	10♂	XY (tripartite) to poles in 1st	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Cincindella vulgaris</i>	22 spg			Trilobed XY in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101

f. Coccinellidae

<i>Adalia bipunctata</i>	20 spg	10♂	10♂	XY to poles in 1st	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Epilachna borealis</i>	18 spg	9♂	9♂	XY to poles in 1st	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Coccinellidae</i> , (a number of sp).....	20 spg	10♂	10♂	XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101

g. Dytiscidae

<i>Colymbetes fuscus</i>	35-37 oog				Gunther, '10	Zool. Jahrb., 30, p. 301
<i>Cybister roselii</i>	ca. 22 spg	12♂	12♂	6♂	Voinov, '03	Arch. Zool. exp. et gen. ser. IV, vol. 1, p. 173
<i>Dytiscus</i>	ca 40 oog ca. 40 ♀ som				Giardina, '01	Intern. Monats. Anat. u. Phys., 18, p. 417
<i>Dytiscus circumcinctus</i> ..	38 spg	19♂	19♂	19♂	Schäfer, '07	Zool. Jahrb., 23, p. 535
<i>Dytiscus marginalis</i>	ca. 40 spg (36-41)			No X	Henderson, '07	Zeit. wiss. Zool., 87, p. 644
<i>Dytiscus marginalis</i>	38 spg	19♂	19♂	X divides in both divisions. 2X in spg.	Schäfer, '07	Zool. Jahrb., 23, p. 535
<i>Dytiscus marginalis</i>	40 oog				Debaisieux, '09	La Cellule, 25, p. 207

h. Elateridae

<i>Elater</i> sp? I.....	19 spg 20 oog	10♂		X to pole in 1st	Stevens, '06	Carnegie Inst., Pub., 36, II, p. 33
<i>Elater</i> sp? II.....	19 spg			X	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Limoneus griseus</i>	17 spg	9♂	8, 9♂	X to pole in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101

II. ARTHROPODA—Continued
 i. Hydrophilidae

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	TYD	REMARKS	OBSERVER	REFERENCE
<i>Hydrophilus piceus</i>		10-12♂	5-6♂				
<i>Hydrophilus piceus</i>	16 spg	16♂ (= 32 el)	8♂ (= 16 el)	8♂	Tetrads and dyads = separate ele- ments	Carnoy, '85 Vom Rath, '92	La Cellule 1, p. 189 Arch. mikr. Anat., 40, p. 102
<i>Hydrophilus piceus</i>	30 som	15♂(gemini)	15♂		XY to poles in 1st	Arnold, '08	Arch. Zellf., 2, p. 181
<i>Chrysocetus auratus</i>		13♂				Stevens, '09	Jour. Exp. Zool., 6, p.
<i>Chrysomela similis</i>		12♂	11, 12♂	11, 12♂	X to pole in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Coptocycla aurichalcea</i>	22 spg	11♂	11♂	11♂	XY to poles in 1st	Nowlin, '06	Jour. Exp. Zool., 3, p. 583
<i>Coptocycla clavata</i>	18 spg				XY	Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Coptocycla guttata</i>	18 spg	9♂	9♂	9♂	XY to poles in 1st	Nowlin, '06	Jour. Exp. Zool., 3, p. 583
<i>Crioceris asparagi</i>		8♀	8♀			Honking, '92	Zeit. wiss. Zool., 54, p. 1
<i>Diabrotica 12-punctata</i> . <i>Diabrotica soror</i>	19 spg	10♂	9, 10♂	9, 10♂	X to pole in 1st		
	20 spg	11♂	9, 10, 11♂	9, 10, 11♂	X; S to pole in 1st or 2nd		
	21 spg	12♂	9-12♂	9-12♂	X; 2S		
		13♂	9-13♂		X; 3S		
<i>Diabrotica vittata</i>	21 spg	11♂	10, 11♂	10, 11♂	X to pole in 1st	Stevens, '08	Jour. Exp. Zool., 5, p. 453
<i>Diabrotica vittata</i>	21, 22 som					Stevens, '08	Jour. Exp. Zool., 5, p. 453
<i>Donacia (sericea?)</i>			15♀			Hoy, '14	Biol. Bull., 27, p. 45
						Henking, '92	Zeit. wiss. Zool., 54, p. 1
<i>Doryphora elvirelis</i>		17♂	17♂		XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Doryphora decemlineata</i> .	36 spg	18♂	18♂			Stevens, '06	Carnegie Inst. Pub 36, II, p. 33

<i>Halica chalybea</i>	22 spg	11♂	16♂	16, 18♂	XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Lema trilineata</i>	32 spg				XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Leptinotarsa signaticollis</i> ..		17♂	16, 17♂		Bivalent X to pole in 1st. Parts separate in 2nd anaph.	Wieman, '10	Jour. Morph., 21, p. 135
<i>Lina laponica</i>		17♂			XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Odontota dorsalis</i>	16 spg 16♂ som	8♂	8♂		XY to poles in 1st. One testis, Y = 2 elements, 17 spg., 8, 9 2nd cyte	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Trihabda canadense</i>	30 spg 30♀ som	15♂	15♂		XY to poles in 1st XX in ♀ diploid	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Trihabda virgata</i>	28 spg, 28♂ som 28♀ som	14♂	14♂		XY to poles in 1st XX in ♀ diploid	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33

j. Lampyridae

<i>Ellychnia corrusca</i>	19 spg				X	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Lampyrus splendidula</i>		6-8♀	6-8♀			Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Photinus consanguineus</i> <i>Photinus pennsylvanicus</i>	19 spg 20 oog 19♂ som	10♂	10♂	9, 10♂	X to pole in 2nd	Henking, '92	Zeit. wiss. Zool. 54, p. 1
						Stevens, '09	Jour. Exp. Zool., 6, p. 101

k. Melandryidae

<i>Penthe obliquata</i>	16 spg		8♂		XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
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l. Meloidae

<i>Epicauta cinerea</i>	20 spg	10♂	10♂		XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Epicauta pennsylvanica</i>							

II. ARTHROPODA—Continued

m. Scarabaeidae

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Euphoria inda</i>	20 spg	10♂	10♂	10♂	XY to poles in 1st	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33
<i>Oryctes nasicornis</i>	12 spg	6♂	6♂	6♂		Prowazek, '02	Arb. Zool. Inst. Wien, 13, p. 223

n. Silphidae

<i>Necrophorus sayi</i>	13 spg	7♂	6, 7♂	6, 7♂	X to pole in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Silpha americana</i>	40 spg	20♂	20♂		XY to poles in 1st	Stevens, '06	Carnegie Inst. Pub., 36 II, p. 33
<i>Silpha carinata</i>	32 spg	10♂ (= 64 el)	10♂ (= 32 el)	16♂		Holmgren, '02	Anat. Anz., 22, p. 194

o. Staphylinidae

<i>Listrotropus cingulatus</i> ...	26 spg	13♂	13♂		XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101
Rove-beetle, brown.....	28 spg	14♂	14♂			Stevens, '09	Jour. Exp. Zool., 6, p. 101
<i>Staphylinus violaceus</i> ...		22♂	22♂		XY to poles in 1st	Stevens, '09	Jour. Exp. Zool., 6, p. 101

p. Tenebrionidae

<i>Blaps hispanica</i>	35 spg (33 in some)	17♂ (16 some)	17, 17 + X♂ (16 in some)		X to pole in 1st. X attached to an- other pair in 1st and 2nd	Fernández-Noni- dez, '14	Trab. Mus. Nac. de C. Nat. de Madrid, Ser., Zool., No. 18, p. 1
<i>Tenebrio molitor</i>	20 spg 20 oeg 20♂ spg 20 ♀ som	10♂	10♂	10♂	XY to poles in 1st XY in spg XX in oeg XX in oeg	Stevens, '05	Carnegie Inst. Pub, 36, p. 1

3. *Diptera*

a. Anthomyiidae

<i>Phorbia brassica</i>	♂♂		XY to poles in 1st	Stevens, '48	Jour. Exp. Zool., 5, p. 359
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b. Cecidomyiidae

<i>Miositor americana</i>	20-24 ♀		Paedogenetic. polar body	Hegner, '14	Jour. Morph., 25, p. 375
<i>Miositor metralbas</i>	20-24 ♀ 20-24 oog. 10-11 late cl		Paedogenetic. polar body	Kahle, '08	Zoologica, 21, p. 1

c. Chironomidae

<i>Chironomus confinis</i> { <i>Chironomus riparius</i>}	4-8 ♀			Hasper, '11	Zool. Jahrb., 31, p. 543
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d. Culicidae

<i>Anopheles punctipennis</i>	♂ spg 6 oog	3♂	3♂	3♂	XY in ♂ attached to another pair. 10 poles in 1st. XX in ♀ at- tached to another pair.	Stevens, '11	Biol. Bull., 20, p. 109
<i>Anopheles</i> sp?.....		6♂	6♂		XY to poles in 1st	Stevens, '11	Biol. Bull., 20, p. 109
<i>Culex pipiens</i>	♂ spg 6 oog	3♂	3♂	3♂	No XY. (= pun- gens? '10)	Stevens, '10 Stevens, '11	Jour. Exp. Zool., 8, p. 207 Biol. Bull., 20, p. 109
<i>Culex pipiens</i>	♂ spg (= 3 pairs) 3 and 6 oog 3 som	3♂	3♂	3♂		Lomen, '14	Jen. Zeits., 45, p. 567
<i>Culex pipiens</i>	3 spg 6 oog 3 som	3♂	3♂	3♂		Taylor, '14	Q. J. M. S., 60, p. 377
<i>Culex tarsalis</i>	♂ spg 6 oog	3♂	3♂	3♂	No XY	Stevens, '11	Biol. Bull., 20, p. 109
<i>Theobaldia incidens</i>	♂ spg 6 oog	3♂	3♂	3♂	No XY	Stevens, '11	Biol. Bull., 20, p. 109

II. ARTHROPODA—Continued

c. Muscidae

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Calliphora vomitoria</i>	12 spg 12 oog	6♂	6♂	6♂	XY to poles in 1st	Stevens, '08	Jour. Exp. Zool., 5, p. 359
<i>Lucilia caesar</i>		6♂	6♂		XY to poles in 1st	Stevens, '08	Jour. Exp. Zool., 5, p. 359
<i>Musca domestica</i>	12 spg 12 oog	6♂	6♂	6♂	XY to poles in 1st	Stevens, '08	Jour. Exp. Zool., 5, p. 359

f. Muscidae acalyptiratae

<i>Drosophila amoena</i>	8 spg 8 oog		4♂		XY in ♂ diploid XX in ♀ diploid	Metz, '14	Jour. Exp. Zool., 17, p. 45
<i>Drosophila ampelophila</i> ...	8 spg 8 oog 8 cl	4♂	4♂	4♂	XY to poles in 1st X may be of two parts	Stevens, '08	Jour. Exp. Zool., 5, p. 359
<i>Drosophila ampelophila</i> ...	8 oog				XX in ♀ diploid	Metz, '14	Jour. Exp. Zool., 17, p. 45
<i>Drosophila funebris</i>	10 or 12 oog				Pair m-chroms, pres- ent (= 12) or ab- sent (= 10)	Metz, '14	Jour. Exp. Zool., 17, p. 45
<i>Drosophila quinaria</i>	8 oog				XX in ♀ diploid	Metz, '14	Jour. Exp. Zool., 17, p. 45
<i>Drosophila repleta</i>	12 oog				XX in ♀ diploid	Metz, '14	Jour. Exp. Zool., 17, p. 45
<i>Drosophila tripunctata</i> ...	8 oog				XX in ♀ diploid	Metz, '14	Jour. Exp. Zool., 17, p. 45
<i>Drosophila</i> 2 sp?	8 oog				XX in ♀ diploid	Metz, '14	Jour. Exp. Zool., 17, p. 45
<i>Drosophila</i> 2 sp? (A and B).....	12 oog				XX in ♀ diploid	Metz, '14	Jour. Exp. Zool., 17, p. 45
<i>Drosophila</i> sp? (C).....	10 oog			5♂	XX in ♀ diploid	Metz, '14	Jour. Exp. Zool., 17, p. 45

<i>Scatophaga pallida</i>	12 spg 12 oog	6♂	6♂	6♂	XY to poles in 1st	Stevens, '08	Jour. Exp. Zool., 5, p. 359
<i>Tetanocera sparsa</i>	12 spg 12 oog	6♂	6♂	6♂	XY to poles in 1st	Stevens, '08	Jour. Exp. Zool., 5, p. 359
g. Sarcophagidae							
<i>Sarcophaga sarracinae</i> ...	12 spg 12 ♀ som	6♂	6♂	6♂	XY to poles in 1st	Stevens, '08	Jour. Exp. Zool., 5, p. 359
h. Syrphidae							
<i>Eristalis tenax</i>	12 spg 12♂ som 12 ♀ som	6♂	6♂	6♂	XY to poles in 1st	Stevens, '08	Jour. Exp. Zool., 5, p. 359
4. Hemiptera							
a. Heteroptera							
1. Belostomatidae							
<i>Zaitia</i> sp? (fluminea or aurantiaceum).....	24 spg	13♂	12♂	12♂	XY to poles in 2nd	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
2. Capsidae							
<i>Calocoris rapidus</i>	30 spg	16♂	15, 16♂		2X, one to pole in 1st, other in 2nd	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Leptoterna dolabrata</i>		17♂				Montgomery, '01	Trans. Amer. Phil. Soc., 20, p. 154
<i>Lygus pratensis</i>	prob. 35 spg	19♂	17, 18♂	17, 18♂	X to pole in 1st, XY to poles in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Proc. Acad. Nat. Sci. Phila., 38, p. 261 Trans. Amer. Phil. Soc., 21, p. 97
<i>Poecilopsaps goniphorus</i> .		18♂	17♂	17♂	XY to poles in 2nd	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Poecilopsaps lineatus</i>		18♂				Montgomery, '01	Trans. Amer. Phil. Soc., 20, p. 154

II. ARTHROPODA—Continued
 3. Coreidae

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2nd -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Alydus curinus</i>	13 spg	7♂	7♂	6, 7♂	X to pole in 2nd	Montgomery, '01	Trans. Amer. Phil. Soc., 20, p. 154
<i>Alydus pilosulus</i>	13 spg	7♂	7♂	6, 7♂	X to pole in 2nd. From correction in '06	Montgomery, '04 Montgomery, '06	Biol. Bull., 6, p. 137 Trans. Amer. Phil. Soc., 21, p. 97
<i>Alydus pilosulus</i>	13 spg 14 oog	7♂	7♂	6, 7♂	X to pole in 2nd	Montgomery, '01	Trans. Amer. Phil. Soc., 20, p. 154
<i>Anasa armigera</i>	21 spg	11♂			X to pole in 2nd From correction in '06	Montgomery, '06	Trans. Amer. Phil. Soc., 21, p. 97
<i>Anasa armigera</i>	22 oog				XX in oog	Wilson, '09	Jour. Exp. Zool., 2, p. 507; 3, p. 1
<i>Anasa tristis</i>	22 spg	11♂	11♂	10, 11♂	Pair small chroms. in spg; to pole in 2nd and degener- ate	Paulmier, '98 Paulmier, '99	Trans. Amer. Phil. Soc., 20, p. 154
<i>Anasa tristis</i>	21 spg 22 oog	11♂	11♂	10, 11♂	X to pole in 2nd. From correction in '06	Montgomery, '01 Montgomery, '04 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Biol. Bull., 6, p. 137 Trans. Amer. Phil. Soc., 21, p. 97
<i>Anasa tristis</i>	21 spg 22 oog	11♂	11♂	10, 11♂	X to pole in 2nd	Wilson, '05, '06	Jour. Exp. Zool., 2, pp. 371 and 507; 3, p. 1
<i>Anasa tristis</i>	22 spg	11♂	11♂	11♂	No accessory but nucleolus	Wilson, '07 Wilson, '11 Foot and Stro- bell, '07 Foot and Stro- bell, '07	Science, 25, no. 631, p. 191 Jour. Morph., 22, p. 71 Biol. Bull., 12, p. 119 Amer. Jour. Anat., 7, p. 279

<i>Anasa tristis</i>	21 spg			10, 11♂	X to pole in 2nd	Lefevre and McGill, '08	Amer. Jour. Anat., 7, p. 469
<i>Anasa tristis</i>	21 spg, 21♂ cl 22 oog, 22 ♀ cl (One cl 23)	11 ♀	11 ♀		X in ♂ diploid XX in ♀ diploid	Morrill '10	Biol. Bull., 19, p. 79
<i>Anasa tristis</i>	21 spg 22 oog	11♂ ⁷ (12 in two cases)	11♂ ⁸		X to pole in 2nd	McClung and Pinney, '10	Kansas Univ. Sci. Bull., 5, p. 349
<i>Anasa tristis</i>	21, 22 som					Hoy, '14	Biol. Bull., 27, p. 45
<i>Anasa</i> sp? (from Cali- fornia).....	21 spg 22 oog	11♂ ⁷			X From correc- tion in '06	Montgomery, '01	Trans. Amer. Phil. Soc., 20, p. 154
<i>Archimerus alternatus</i> (= calcarator?).....	15 spg 16 oog 15♂ cl 10 ♀ cl	8♂ ⁷ 8 ♀	7, 8♂ ⁷ 8 ♀		X to pole in 1st (♂) X in ♂ diploid. XX in ♀ diploid.	Montgomery, '04 Montgomery, '06	Biol. Bull., 6, p. 137 Trans. Amer. Phil. Soc., 21, p. 97
<i>Archimerus calcarator</i>	15 spg 16 oog	8♂ ⁷	7, 8♂ ⁷		X to pole in 1st	Morrill, '10	Biol. Bull., 19, p. 79
<i>Catorintha</i>	25 spg				X	Wilson, '07	Science, 25, no. 631, p. 191
<i>Charisterus antennator</i>		13♂ ⁷	13♂ ⁷		X to pole in 2nd	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Charisterus antennator</i> ?	21 spg				X to pole in 2nd. Species may be strongly identifi- fied, labelled by Paulmier	Wilson, '05	Jour. Exp. Zool., 2, pp. 371 and 307 Jour. Exp. Zool., 6, p. 69
<i>Charisterus antennator</i>	25 spg 26 oog				X in spg. XX in oog	Wilson, '09	Jour. Exp. Zool., 6, p. 69
<i>Chelinidea vittigera</i>	21 spg				X	Wilson, '07	Science, 25, no. 631, p. 191
<i>Chelinidea vittigera</i>	21 spg 22 oog 21♂ cl 22 ♀ cl (one spg 22)				X in ♂ ⁷ diploid XX in ♀ diploid	Morrill, '10	Biol. Bull., 19, p. 79

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Corizus alternatus</i>	13 spg	7♂ ³ (8 if 2 uni- valent)	7♂	6, 7♂	X to pole in 2nd. From correction in '06	Montgomery, '01 Montgomery, '04 Montgomery, '06	Proc. Acad. Nat. Sci. Phila., 53, p. 261 Biol. Bull., 6, p. 137 Trans. Amer. Phil. Soc., 21, p. 97
<i>Corizus lateralis</i>		7♂ ¹	7♂	6, 7♂	X to pole in 2nd	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Corynocoris distinctus</i>	25 spg 26 oeg				X in spg. XX in oog	Wilson, '09	Jour. Exp. Zool., 6, p. 69
<i>Euthoetha galeator</i>	21 spg 22 oeg				X in spg. XX in oog	Wilson, '07 Wilson, '09	Science, 25, no. 631, p. 191 Jour. Exp. Zool., 6, p. 69
<i>Harmostes reflexus</i>	13 spg	7♂ ³ (8 if 2 uni- valent)	7♂	6, 7♂	X to pole in 2nd	Montgomery, '01 Montgomery, '01 Montgomery, '04 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Proc. Acad. Sci. Phila., 53, p. 261 Biol. Bull., 6, p. 137 Trans. Amer. Phil. Soc., 21, p. 97
<i>Harmostes reflexus</i>	13 spg 14 oeg				X to pole in 2nd	Wilson, '06	Jour. Exp. Zool., 3, p. 1
<i>Leptocoris trivittatus</i>	13 spg 14 oeg				X in spg. XX in oog	Wilson, '07 Wilson, '09	Science, 25, no. 631, p. 191 Jour. Exp. Zool., 6, p. 69
<i>Leptoglossus phyllopus</i>	21 spg 22 oeg	11♂			X to pole in 2nd	Wilson, '07 Wilson, '09 Wilson, '11	Science, 25, no. 631, p. 191 Jour. Exp. Zool., 6, p. 69 Jour. Morph., 22, p. 71
<i>Margus inconspicuus</i>	23 spg 24 oeg				X in spg. XX in oog	Wilson, '07 Wilson, '09	Science, 25, no. 631, p. 191 Jour. Exp. Zool., 6, p. 69

		11♂ (1 triad)	11, 12♂	10, 11, 12♂	X to pole in 2nd 1 m to other in 1st	Wilson, '07 Wilson, '09 Wilson, '10	Biol. Bull., 12, p. 303 Jour. Exp. Zool., 6, p. 147 Jour. Exp. Zool., 9, p. 53
Metapodius femoratus	22 spg						
	22 spg 22 oög	12♂	11♂	11♂	XY to poles in 2nd		
	23 spg 23 oög	13♂	11, 12♂	11, 12♂	XY and S to poles in 2nd, S may be attached to X or Y (= 11) or free (= 12) in 2nd met- aphase		
	24 spg 24 oög	14♂	11, 12♂	11, 12, 13♂	XY and 2S (as above)		
	26 spg 26 oög	16♂	11-15♂	11-15♂	XY and 4S (as above)		
	28 oög				XY and 6S? (as above)		
	22 spg 22 oög	12♂	11♂	11♂	XY to poles in 1st	Wilson, '07 Wilson, '09	Biol. Bull., 12, p. 303 Jour. Exp. Zool., 6, p. 147
Metapodius granulatus	23 spg 23 oög	13♂	11, 12♂	11, 12♂	XY and S (as in M. femoratus)		
	24 spg 24 oög	14♂	11, 12♂	11, 12, 13♂	XY and 2S (as in M. femoratus)		
	25 spg 25 oög	15♂	11♂		XY and 3S (as in M. femoratus)		
	26 spg 26 oög	16♂	11-15♂	11-15♂	XY and 4S (as in M. femoratus)		
		17♂	11♂		XY and 5S (as in M. femoratus)		
	21 spg	11♂	11♂	10, 11♂	X to pole in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
	21 spg 22 spg 22 oög	11♂ 12♂	11♂ 11♂	10, 11♂ 11♂	X to pole in 2nd XY to poles in 2nd	Wilson, '07 Wilson, '09	Biol. Bull., 12, p. 303 Jour. Exp. Zool., 6, p. 147
Metapodius terminalis.	23 spg 23 oög	13♂	11, 12♂	11, 12♂	XY and S (as in M. femoratus)		
	24 spg 24 oög	14♂	11, 12♂	11, 12, 13♂	XY and 2 S (as in M. femoratus)		

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Metapodius terminalis</i> .	25 spg 25 oog 26 spg 26 oog	15♂ 16♂	11♂ 11-15♂	11-15♂	XY and 3S (as in M. femoratus) XY and 4S (as in M. femoratus)	Wilson, '07	Science, 25, no. 631, p. 191
<i>Narnia</i>	21 spg				X in spg. XX in oog	Wilson, '07 Wilson, '09 Wilson, '11	Science, 25, no. 631, p. 191 Jour. Exp. Zool., 6, p. 69 Jour. Morph., 22, p. 71
<i>Pachylis gigas</i>	15 spg 16 oog	8♂			X to pole in 2nd	Montgomery, '01 Montgomery, '04 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Biol. Bull., 6, p. 137 Trans. Amer. Phil. Soc., 21, p. 97
<i>Protenor bellifragi</i>	13 spg 14 oog	7♂	7♂	6, 7♂	X to pole in 2nd	Wilson, '05, '06 Wilson, '11	Jour. Exp. Zool., 2, p. 507; 3, p. 1 Jour. Morph., 22, p. 71
<i>Protenor bellifragi</i>	13 spg 14 oog 13♂ cl 14♀ cl	7♀		7 pron.	X in ♂ diploid. XX in ♀ diploid	Morrill, '10	Biol. Bull., 19, p. 79
<i>Syromastes marginatus</i> ..	22 spg 22 ♀ som	11♂	10♂ (= 11, X a pole in metaphase)	10, 11♂	Bivalent X to pole in 2nd. Sperm with 10 not func- tional	Gross, '04 Gross, '04 Gross, '12	Verh. deutsch. Zool. Gesell., '04, p. 180 Zool. Jahrb., 20, p. 439 Zool. Jahrb., Abt. f. Zool., 32, p. 99
<i>Syromastes marginatus</i> ..	22 spg 24 oog	11♂	10♂ (= 12, X at pole in metaphase)	10, 12♂	Double X to pole in 2nd. X = 2 une- qual parts, united in 1st metaphase	Wilson, '09 Wilson, '09	Jour. Exp. Zool., 6, p. 69 Biol. Bull., 16, p. 199
4. Corixidae							
<i>Corixa verticilis</i>		12♂				Montgomery, '01	Proc. Acad. Nat. Sci. Phila., 53, p. 261

5. Galguliidae

Galgulus oculatus (= Gelastocoris).....	35 spg 38 oög 38 ♀ som	20♂	20♂	16, 19♂	XY to poles in 2nd X = 4 elements	Payne, '09	Biol. Bull., 16, p. 119
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6. Hydrometridae

<i>Hydrometra lacustris</i>	11 spg	12♂	12♂		Large chrom. of spg = 2 in spc.	Wilke, '07 Wilke, '13	Jen. Zeits., 35, p. 669 Arch. Zellf., 10, p. 203
<i>Hydrometra paludum</i>	10-15 spg (prob. 12)	12♂ ^a (few with 13 and 14)	12♂		X? divides in both divisions	Wilke, '12 Wilke, '13	Zool. Anz., 40, p. 216 Arch. Zellf., 10, p. 203
<i>Hygotrechus</i> sp?.....	21 spg	11♂	11♂	10, 11♂	X to pole in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Limnotrechus marginatus</i>		11♂	11♂		X to pole in 2nd	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97

7. Lygaeidae

<i>Cymus angustatus</i>			14♂		XY to poles in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Phil. Trans. Amer. Phil. Soc., 21, p. 97
<i>Cymus luridus</i>		15♂				Montgomery, '01	Proc. Acad. Nat. Sci. Phila., 55, p. 261
<i>Ichnodemus falicus</i>	16 spg	9♂	8♂	8♂	XY to poles in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Phil. Trans. Amer. Phil. Soc., 21, p. 97
<i>Lygaeus bicrueis</i>	14 spg 14 oög	8♂	7♂	7♂	XY to poles in 2nd	Wilson, '09 Wilson, '12	Jour. Exp. Zool., 6, p. 68 Jour. Exp. Zool., 13, p. 345
<i>Lygaeus turcicus</i>	14 spg 14 oög	8♂ (occasional- ly 9)	7♂	7♂	XY to poles in 2nd	Wilson, '05 Wilson, '06	Jour. Exp. Zool., 2, pp. 371 and 507 Jour. Exp. Zool., 3, p. 1

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Oedaneala dorsalis</i>	13 spg	7♂ (6 if small chroms. at poles)	7♂	6, 7♂	X to pole in 2nd	Montgomery, '01 Montgomery, '04 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Biol. Bull., 6, p. 137 Trans. Amer. Phil. Soc., 21, p. 97
<i>Oncopeltus fasciatus</i>	16 spg	9♂	8♂	8♂	XY to poles in 2nd	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Oncopeltus fasciatus</i>	16 spg 16 oog	9♂	8♂	8♂	XY to poles in 2nd (Sometimes X and Y equal in size)	Wilson, '09 Wilson, '12	Jour. Exp. Zool., 6, p. 69 Jour. Exp. Zool., 13, p. 345
<i>Peliopelta abbreviata</i>	14 spg	8♂ (9 if small chroms, side by side)	7♂		XY (equal) to poles in 2nd. From cor- rection in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Soc., 21, p. 97
8. Nabidae							
<i>Coriscus ferus</i>		10♂	8♂ (= 9, X and Y at poles in met- aphase)	9♂	XY to poles in 2nd	Montgomery, '01	Trans. Amer. Phil. Soc., 20, p. 154
<i>Nabis annulatus</i>		10♂				Montgomery, '01 Montgomery, '06	Proc. Acad. Nat. Sci. Phila., 53, p. 261 Trans. Amer. Phil. Soc., 21, p. 97
9. Naucoridae							
<i>Palocoris femorata</i>	20? spg					Montgomery, '01	Trans. Amer. Phil. Soc., 20, p. 154
10. Notonectidae							
<i>Notonecta glauca</i>		12-13♂	12♂ (figured)			Pantel and Sinéty '06	La Cellule, 23, p. 89
<i>Notonecta insulata</i>		14♂	12, 13♂ (2 chroms. may be fused or not)	12♂	XY to poles in 2nd	Browne, '10 Browne, '13	Biol. Bull., 20, p. 19 Jour. Exp. Zool., 14, p. 61

	24 spg	13♂	12♂	12♂	12♂	XY to poles in 2nd	Browne, '10 Browne, '13	Biol. Bull., 20, p. 19 Jour. Exp. Zool., 14, p. 61
<i>Notonecta irrorata</i>								
<i>Notonecta undulata</i>	26 spg	14♂	13♂	13♂	13♂	XY to poles in 2nd	Browne, '10 Browne, '13	Biol. Bull., 20, p. 19 Jour. Exp. Zool., 14, p. 61
11. Pentatomidae								
<i>Banasa calva</i>	26 spg 26 oog	14♂ 15♂	13♂ 13, 14♂	13♂ 13, 14♂	13♂ 13, 14♂	XY to poles in 2nd XY to poles in 2nd. S to pole in 1st. From correction in '07	Wilson, '05 Wilson, '07	Jour. Exp. Zool., 2, p. 507 Biol. Bull., 12, p. 303
<i>Banasa dimidiata</i>	16 spg 16 oog	9♂	8♂	8♂	8♂	XY to poles in 2nd	Wilson, '07	Biol. Bull., 12, p. 303
<i>Brochymena</i> sp? ²	14 spg	8♂	7♂	7♂	7♂	XY to poles in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Brochymena</i> sp? ²	14 spg	8♂	7♂	7♂	7♂	XY to poles in 2nd	Wilson, '05	Jour. Exp. Zool., 2, p. 371
<i>Coenus delius</i>	14 spg	8♂	7♂	7♂	7♂	XY to poles in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Coenus delius</i>	14 spg 14 oog	8♂ (occasionally 9)	7♂	7♂	7♂	XY to poles in 2nd	Wilson, '05 Wilson, '06	Jour. Exp. Zool., 2, pp. 371 and 507 Jour. Exp. Zool., 3, p. 1
<i>Cosmopepla carnifex</i>	16 spg	9♂	8♂	8♂	8♂	XY to poles in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Eurygaster alternatus</i>		7♂	6♂	6♂	6♂	XY to poles in 2nd	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Euschistus crassus</i>	12 spg 12 oog	7♂	6♂	6♂	6♂	XY to poles in 2nd	Foot and Strobel, '12	Arch. Zellf., 9, p. 47

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Euschistus fissilis</i>	14 spg 14 oog	8♂	7♂	7♂	XY to poles in 2nd	Wilson, '05	Jour. Exp. Zool., 2, pp. 371 and 507
<i>Euschistus ictericus</i>	14 spg 14 oog				XY in spg. XX in oog	Wilson, '06	Jour. Exp. Zool., 3, p. 1
<i>Euschistus servus</i>	14 spg 14 oog				XY to poles in 2nd	Wilson, '06	Jour. Exp. Zool., 3, p. 1
<i>Euschistus servus</i>	14 spg 14 oog	8♂		7♂	XY to poles in 2nd	Foot and Stro- bell, '14	Jour. Exp. Zool., 3, p. 1
<i>Euschistus tristignus</i>	14 spg	8♂	7♂	7♂	XY to poles in 2nd	Montgomery, '01	Arch. Zellf., 12, p. 485
<i>Euschistus tristignus</i>	14 spg 14 oog	8♂	7♂	7♂	XY to poles in 2nd	Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154
<i>Euschistus variolarius?</i> (= "Pentatoma").	14 spg (13-15)	8♂	7♂ (6-9)	7♂	XY in spg. XX in oog	Wilson, '06	Trans. Amer. Phil. Soc., 21, p. 97
<i>Euschistus variolarius</i>	14 spg 14 oog				XY to poles in 2nd. From correction in '06. Prob. more than one species	Montgomery, '97 Montgomery, '98 Montgomery, '01	Jour. Exp. Zool., 3, p. 1
<i>Euschistus variolarius</i>	14 spg 14 oog	8♂	7♂	7♂	XY to poles in 2nd	Montgomery, '06	Zool. Anz., 20, p. 457 Zool. Jahrb., 12, p. 1
<i>Mineus bioculatus</i>	14 spg 14 oog	8♂	7♂	7♂	XY to poles in 2nd	Montgomery, '10 Montgomery, '11	Trans. Amer. Phil. Soc., 20, p. 154 Arch. Zellf., 5, p. 120 Jour. Morph., 22, p. 731
<i>Mormidea lugens</i>	14 spg	8♂	7♂	7♂	XY to poles in 2nd. X and Y nearly equal	Wilson, '06	Jour. Exp. Zool., 3, p. 1
<i>Nezara hilaris</i>	14 spg	8♂	7♂	7♂	XY to poles in 2nd. From correction in '06	Foot and Stro- bell, '14	Arch. Zellf., 12, p. 485
					XY to poles in 2nd. From correction in '06	Wilson, '06	Jour. Exp. Zool., 3, p. 1
					From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
					From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97

Nezara hilaris	14 spg 14 oog	8♂	7♂	7♂	XY to poles in 2nd. X and Y nearly equal	Wilson, '05 Wilson, '06 Wilson, '11	Jour. Exp. Zool., 2, p. 371 Jour. Exp. Zool., 3, p. 1 Jour. Morph., 22, p. 71
Nezara viridula	14 spg 14 oog	8♂	7♂	7♂	XY to poles in 2nd	Wilson, '05 Wilson, '11	Jour. Exp. Zool., 2, p. 371 Jour. Morph., 22, p. 71
Oebalus pugnax	10 spg 10 oog				XY in spg. XX in oog	Wilson, '09	Jour. Exp. Zool., 6, p. 69
Pentatoma juniperina (= Chlorochroa)	14 spg					Wilson, '13	Biol. Bull., 24, p. 392
Pentatoma senilis (= Rhytidolomia)	6 spg	4♂	3♂	3♂	XY to poles in 2nd	Wilson, '13	Biol. Bull., 24, p. 392
Pentatoma see Euschis- tus variolarius	14 spg	8♂	7♂	7♂	XY to poles in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
Peribolus limbolaris						Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
Perillus confluentus	14 spg	8♂	7♂	7♂	XY to poles in 2nd. From correction in '06	Wilson, '09	Proc. 7th Intern. Zool. Cong., Boston, 1907, p. 1
Podisus bracteatus..... Podisus crocatus.....	14 spg					Wilson, '09	Proc. 7th Intern. Zool. Cong., Boston, 1907, p. 1
Podisus modestus..... Podisus placidus.....	16 spg					Wilson, '09	Proc. 7th Intern. Zool. Cong., Boston, 1907, p. 1
Podisus spinosus (= ma- culiventris)	16 spg 16 oog	9♂	8♂	8♂	XY to poles in 2nd. From correction in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
Podisus spinosus.....	16 spg 16 oog	9♂	8♂	8♂	XY to poles in 2nd	Wilson, '05 Wilson, '06	Jour. Exp. Zool., 2, p. 371 Jour. Exp. Zool., 3, p. 1
Stiretrus anchorago	14 spg 14 oog				XY in spg. XX in oog	Wilson, '09	Jour. Exp. Zool., 6, p. 69

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTIENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Thyanta culcata</i>	27 spg 28 oog	15♂			XY to poles in 2nd X = 2 elements united linearly in 2nd	Wilson, '09 Wilson, '11	Proc. 7th Inter. Cong. Boston, 1907, p. 1 Jour. Morph., 22, p. 71
<i>Thyanta custator</i>	16 spg 16 oog				XY to poles in 2nd	Wilson, '11	Jour. Morph., 22, p. 71
<i>Trichopepla semivittata</i> ..	16 spg	8♂	7♂	7♂	XY to poles in 2nd Small chroms. of diploid usually lacking in haploid	Montgomery, '01 Montgomery, '04 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Biol. Bull., 6, p. 137 Trans. Amer. Phil. Soc., 21, p. 97
<i>Trichopepla semivittata</i> ..		8, 9♂	7♂		XY to poles in 2nd Small chrom. may be lacking in 1st (= 8). Un- certain case	Wilson, '05	Jour. Exp. Zool., 2, p. 371
12. Phymatidae							
<i>Phymata wolffi</i> ?.....	29? spg	15♂			X? From correc- tion in '06	Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Phil. Trans. Amer. Soc., 21, p. 97
13. Pyrochoridae							
<i>Largus cinctus</i>	11 spg 12 oog				X in spg. XX in oog	Wilson, '07	Science, 25, no. 631, p. 191 Jour. Exp. Zool., 6, p. 68
<i>Largus succintus</i>	13 spg 14 oog				X in spg. XX in oog	Wilson, '09 Wilson, '07 Wilson, '09	Science, 25, no. 631, p. 191 Jour. Exp. Zool., 6, p. 69
<i>Pyrochorosis apterus</i>	24 spg 24 oog 24 ♀ som 24 cl	12♂ 12♀	12♂ 12♀	11, 12♂ 12♀	X to pole in 2nd♂ (= chromatin nu- cleolus)	Henking, '90 Henking, '91 Henking, '92	Intern. Monats. Anat. u. Phys., 7, p. 243 Zeits. wiss. Zool., 51, p. 685 Zeits. wiss. Zool., 54, p. 1

Pyrrochoris apterus.....	24 spg 24 oog 24 som	12♂.	12♂	11, 12♂ (those with 11 de- generate)	X to pole in 2nd. X = 2 elements, separate in di- ploid, united in haploid X to pole in 2nd	Gross, '06 Gross, '12	Zool. Jahrb., 23, p. 269 Zool. Jahrb., Abt. f. Zool., 32, p. 99
	23 spg 24 oog	12♂	12♂	11, 12♂		Wilson, '09 Wilson, '09	Jour. Exp. Zool., 6, p. 69 Biol. Bull., 16, p. 199
14. Reduviidae							
Acholla ampliata (= multispinosa).....	32 spg	16♂	16♂	16♂		Montgomery, '01 Montgomery, '06 Montgomery, '10	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97 Arch. Zellf., 5, p. 120 (note)
Acholla ampliata.....		16♂				Payne, '10	Biol. Bull., 18, p. 174
Acholla multispinosa (= Sinea diadema).....		16♂	16♂	16♂	3 chroms. associ- ated in 1st	Montgomery, '01 Montgomery, '06 Montgomery, '10	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97 Arch. Zellf., 5, p. 120 (note)
Acholla multispinosa.....	26 spg 30 oog	16♂	16♂	11, 15♂	XY to poles in 2nd X = 5 elements	Payne, '10	Biol. Bull., 18, p. 174
Apiomeris crassipes.....	24 spg	13♂	12♂	12♂	XY to poles in 2nd	Payne '12	Jour. Morph., 23, p. 331
Conorhinus sanguisugus..	23 spg	13♂	13♂	11, 12♂	XY to poles in 2nd X = 2 elements	Payne, '09 Payne, '12	Biol. Bull., 16, p. 119 Jour. Morph., 23, p. 331
Diplocodus exsanguis.....	26♂ som 26♀ som	14♂	13♂	13♂	XY to poles in 2nd	Payne, '09	Biol. Bull., 16, p. 119
Fitchia spinosula.....	27 spg 28 oog	15♂	15♂	13, 14♂	XY to poles in 2nd X = 2 elements	Payne, '09	Biol. Bull., 16, p. 119
Phaenontis modesta.....	25 spg	15♂	15♂	11, 14♂	XY to poles in 2nd X = 4 elements	Payne, '12	Jour. Morph., 23, p. 331

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Prionidus cristatus</i>	26 spg					Montgomery, '01 Montgomery, '06	Trans. Amer. Phil. Soc., 20, p. 154 Trans. Amer. Phil. Soc., 21, p. 97
<i>Prionidus cristatus</i>	26 spg 28 oog	15♂	15♂	12, 14♂	XY to poles in 2nd X = 3 elements	Payne, '09	Biol. Bull., 16, p. 119
<i>Pseliodes cinctus</i>	28 spg 30 oog	16♂	16♂	13, 15♂	XY to poles in 2nd X = 3 elements	Payne, '12	Jour. Morph., 23, p. 331
<i>Reduvius personatus</i>		12♂	11♂		XY to poles in 2nd	Payne, '12	Jour. Morph., 23, p. 331
<i>Rocconata annulicornis</i> ...	27 spg	15♂	15♂	13, 14♂	XY to poles in 2nd X = 2 elements.	Payne, '09	Biol. Bull., 16, p. 119
<i>Sinea complexa</i> } <i>Sinea confusa</i> } <i>Sinea diadema</i> } <i>Sinea spinipes</i> }	28 spg 30 oog	16♂	16♂	13, 15♂	XY to poles in 2nd X = 3 elements	Payne, '09 Payne, '12	Biol. Bull., 16, p. 119 Jour. Morph., 23, p. 331
<i>Sinea rileyi</i>		18♂	18♂	13, 17♂	XY to poles in 2nd X = 5 elements	Payne, '12	Jour. Morph., 23, p. 331

15. Tingitidae							
<i>Tingis clavata</i>		7♂	7♂ (8 in one case, X and Y in same cell)	7♂	XY to poles in 1st	Montgomery, '01 Montgomery, '06	Proc. Acad. Nat. Sci. Phila., 53, p. 261 Trans. Amer. Phil. Soc., 21, p. 97

b. Homoptera							
1. Aphidae							
Aphid. bearberry = <i>Phyllaphis coveni</i>	5 spg 6 oog	3♂. One cyst had 6 (2X)	2, 3♂. Cells with 2 de- generate. One cyst had 4, 6 or 5♂	3♂	X to pole in 1st. One cyst had 2X, to pole in 1st or one X to each pole	Morgan, '15	Jour. Exp. Zool., 19, p. 285

Aphid, beech (wooly).....		8♂	7, 8♂		X to pole in 1st. From correction in '09	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115
Aphid, birch, paper.....		9♂				Stevens, '06	Carnegie Inst. Pub., 51, p. 1
Aphid, clover.....		8♂				Stevens, '06	Carnegie Inst. Pub., 51, p. 1
Aphid, goldenrod, beach..	12 pa cl	6♂	5, 6♂		X to pole in 1st. From correction in '09	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115
Aphid, goldenrod, tall = solidago altissima.....		6♂	5, 6♂		X to pole in 1st. From correction in '09	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115
Aphid, gouni.....	10 cl (winter egg)	5 ♀		5♂ pron 5 ♀ pron		Stevens, '06	Carnegie Inst. Pub., 51, p. 1
Aphid, maple.....		16♂	15, 16♂		X to pole in 1st	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115
Aphid, milkweed (black).	8 spg 8 pa cl	4♂ 8 pa ♀	3, 4 ♀		X to pole in 1st. From correction in '09	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115
Aphid, milkweed (or- ange).....	8 pa cl	7, 8 pa ♀			May be ♂ and ♀ producing	Stevens, '06	Carnegie Inst. Pub., 51, p. 1
Aphid, milkweed (pale)..		7♂	7♂		One individual had 9 in 1st; probably different species	Stevens, '09 Stevens, '06	Carnegie Inst. Pub., 51, p. 1 115
Aphid, nasturtium.....		4♂	3, 4♂		May be black milk- weed	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115
Aphid, oak, red I.....		7♂	7♂			Stevens, '06	Carnegie Inst. Pub., 51, p. 1
Aphid, oak, red II.....		8♂				Stevens, '06	Carnegie Inst. Pub., 51, p. 1

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1ST -CYTE	2ND -CYTE	TID	REMARKS	OBSERVER	REFERENCE
Aphid, oak (white).....		7♂				Stevens, '06	Carnegie Inst. Pub., 51, p. 1
Aphid oenothera I = Aphis oenotherae.....	10 spg 10 pa el 9♂ embryo	5♂ 5♀ 9♂ egg 10 pa ♀	4, 5♂	4, 5♂	X to pole in 1st. One chrom. goes to p.b. of ♂ egg. From correction in '09	Stevens, '05 Stevens, '05 Stevens, '06 Stevens, '09 Stevens, '10 Stevens, '06 Stevens, '06	Carnegie Inst. Pub., 36, p. 1 Jour. Exp. Zool., 2, p. 313 Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115 Biol. Bull. 18, p. 72 Carnegie Inst. Pub., 51, p. 1 Carnegie Inst. Pub., 51, p. 1
Aphid, oenothera II.....		4♂ 8 pa ♀ 8 pa ♀	4♂	4♂		Stevens, '06	Carnegie Inst. Pub., 51, p. 1
Aphid, pea.....	8 pa el				One polar body	Stschelkanovzew, '04	Biol. Centr., 24, p. 104
Aphis rosae.....	8 + 3 double pa el	14♀	10♀		One p.b. in pa eggs. Chroms introduced	Hewitt, '06	Mem. and Proc. Manchester Lit. and Phil. Soc., 50, no. 6, p. 1
Aphis rosae.....		10♀					
Aphis rosae = (brown) rose.....	10 spg 10 pa el	5♂ 5♀ 10 pa ♀	5♂ 5♀		2 p.b.s. in winter egg and reduction. 1 p.b. in pa egg and no reduction	Stevens, '05 Stevens, '06	Jour. Exp. Zool., 2, p. 313 Carnegie Inst. Pub., 51, p. 1
Aphis rosae.....	10 pa el 10 pa som ♀	10♀				Von Baehr, '09	Arch. Zellf., 3, p. 289
Aphid, rose (green).....	14 pa el	7♂ 14 pa ♀	6, 7♂		X to pole in 1st. From correction in '09	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool. 6, p. 115
Aphid, rose (migratory).....	18 el (winter egg)	9♀				Stevens, '06	Carnegie Inst. Pub., 51, p. 1

<i>Aphis saliceti</i>	5 spg 5♂ som 6♀ som 6 pa ♀ cl	3♂ 6♀. One case, 5	2, 3♂. Those with 2 de- generate	3♂	X to pole in 1st	Von Baehr, '08 Von Baehr, '09 Von Baehr, '12	Zool. Anz., 33, p. 507 Arch. Zellf., 3, p. 269 La Cellule, 17, p. 383
<i>Aphis salicicola</i>	5 spg 6 pa cl	3♂ 6 pa ♀	2, 3♂. Those with 2 de- generate	3♂	X to pole in 1st	Morgan, '09	Jour. Exp. Zool., 7, p. 239
<i>Aphis salicicola</i> (= Harps- well willow).....	5 spg 6 pa cl	3♂ 6 pa ♀	2, 3♂		X to pole in 1st. From correction in '09	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115
<i>Aphid, star cucumber</i>		5♂	4, 5♂		X to pole in 1st From correction in '09	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115
<i>Aphid, willow, Harps- well, see Aphis salicicola</i>		5♂	4, 5♂		X to pole in 1st. From correction in '09	Stevens, '06 Stevens, '09	Carnegie Inst. Pub., 51, p. 1 Jour. Exp. Zool., 6, p. 115
<i>Aphid, willow, Sarinac</i> ...		10♂				Morgan, '09	Jour. Exp. Zool., 7, p. 239
<i>Chaetophorus viminalis</i> ...		4♂	3, 4♂. Those with 3 de- generate		X to pole in 1st	Morgan, '09	Jour. Exp. Zool., 7, p. 239
<i>Lachnus dentatus</i>		3♂ 3♀ 6 pa ♀	3♂ 3♀	3♂ pron 3♀ pron	One p.b. in pa	Tanner, '07	Zool. Jahrb., 24, p. 609
<i>Melanoxanthus salici- cola</i> <i>Melanoxanthus salicis</i>	6 spg 6 oog 6 pa ♀ cl	3♂ 3♀ 6 pa ♀	3♂ 3♀			Von Baehr, '08 Von Baehr, '09	Zool. Anz., 33, p. 507 Arch. Zellf., 3, p. 269
<i>Pemphigus pyritiformis</i> .. <i>Pemphigus spirotheca</i> }	20 pa ♀ som	20 ♀					
<i>Phyllaphis cowneni</i> , see <i>Aphid, bearberry</i>							
<i>Phylloxera caryacaulis</i> ...	5(=6) spg 6(=8) oog	3(=4)♂ 3(=4)♀ 6(=8)♀ pa	3(=4)♂, 2♂ 3(=4)♀ Spe with 2 de- generate	3♂ = 4♂ 3♀ = 4♀ 5♂ pa egg 6♀ pa egg	Double X to pole in 1st. Double X to p.b. in pa ♂ producers. One p.b. in pa line.	Morgan, '08 Morgan, '09 Morgan, '09 Morgan, '12 Morgan, '15	Proc. Soc. Exp. Biol. and Med., 3, p. 56 Science, 24, p. 234 Jour. Exp. Zool., 7, p. 239 Jour. Exp. Zool., 12, p. 479 Jour. Exp. Zool., 19, p. 289

II. ARTHROPODA—Continued

SPECIES	D'PLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTN	-TID	REMARKS	OBSERVER	REFERENCE
<i>Phylloxera carynefoliae</i>	8 egg of mi- grant					Morgan, '09	Jour. Exp. Zool., 7, p. 239
<i>Phylloxera caryaeglobuli</i> .	22♂ eggs 22♀ eggs					Morgan, '06 Morgan, '09	Biol. Bull., 10, p. 201 Jour. Exp. Zool., 7, p. 239
<i>Phylloxera depressa</i>	6 egg of stem mother					Morgan, '09	Jour. Exp. Zool., 7, p. 239
<i>Phylloxera fallax</i>	10 spg 10 oeg 10♀ som	6♂ 6♀ 12 pa♀	4, 6♂ 6♀ Spg with 4 de- generate	6♂ 6♀ 10♂ pa egg 12♀ pa egg	2X to pole in 1st; may be united. 2X to p.b. in pa ♂ producers. One p.b. in pa line	Morgan, '06 Morgan, '09 Morgan, '09 Morgan, '12 Morgan, '15	Biol. Bull., 10, p. 201 Science, 29, p. 234 Jour. Exp. Zool., 7, p. 239 Jour. Exp. Zool., 12, p. 479 Jour. Exp. Zool., 19, p. 285
<i>Phylloxera globosum</i>	6 egg of stem mother 6♂ eggs 6♀ eggs					Morgan, '06 Morgan, '09	Biol. Bull., 10, p. 201 Jour. Exp. Zool., 7, p. 239
<i>Phylloxera subelliptica</i> ...	6 egg of mi- grant					Morgan, '09	Jour. Exp. Zool., 7, p. 239
<i>Schizoneura lanigera</i> } <i>Schizoneura ulmi</i>	12 pa♀ som	12♀ (11 in 2 causes)				Von Baehr, '08 Von Baehr, '09	Zool. Anz., 33, p. 507 Arch. Zellf., 3, p. 269
2. Ceropidae							
<i>Aphrophora parallela</i>		15♂	14, 15♂		X to pole in 1st	Boring and Fog- ler, '15	Biol. Bull., 29, p. 312
<i>Aphrophora quadrangu- laris</i> (= <i>Lepronia</i> <i>quad</i>).....	21 spg	11♂ 12♂	10, 11♂ 11, 12♂	10, 11♂	X to pole in 1st. Probably 2 spe- cies	Boring, '07 Boring, '13	Jour. Exp. Zool., 4, p. 469 Biol. Bull., 24, p. 133
<i>Aphrophora quadrangu- laris</i> (Harpsswell).....	23 spg 24 oeg 23♂ som	12♂	11, 12♂	11, 12♂	X to pole in 1st	Stevens, '06	Carnegie Inst. Pub. no. 36, II, p. 33

Aphrophora quadrinotata	14♂	13, 14♂	13, 14♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
Aphrophora spumaria....	6-12♂				Carnoy, '85	La Cellule, 1, p. 189
Aphrophora spumaria....	12♂			X to pole in 1st	Boring, '13	Biol. Bull., 24, p. 133
Clastoptera obtusa.....	15 spg 8♂	7, 8♂	7, 8♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
Clastoptera proteus.....	7♂			X to pole in 1st	Boring and Fogler, '15	Biol. Bull., 29, p. 312
Lepronia quadrangulans, see Aphrophora quadrangularis						
Philaenus lineatus.....	29 spg 15♂	14, 15♂		X to pole in 1st	Boring and Fogler, '15	Biol. Bull., 29, p. 312
Philaenus spumarius.....	23 spg 24 oeg 23♂ som 24 ♀ som	12♂	11, 12♂	X to pole in 1st	Boring, '13	Biol. Bull., 24, p. 133

3. Cicadidae						
Cicada tibicen.....	12 spg	6♂ (= 24 el)			Wilcox, '95	Bull. Mus. Comp. Zool. Harvard, 21, p. 3

4. Coecidae						
Icerya purchasi.....	4 spg 4 oeg 4 som	2♂ 2♀	2♂ 2♀		Pierantoni, '12 Pierantoni, '14	Archivo Zoologico, 5, p. 321 Archivo Zoologico, 7, p. 27

5. Fulgoridae						
Amphiscepa bivittata....	25 spg	13♂	12, 13♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
Poeciloptera bivittata....		13♂	12, 13♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
Poeciloptera pruinosus Poeciloptera septentrionalis	27 spg 28 ♀ som	14♂	13, 14♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469

II. ARTHROPODA—Continued

6. Jassidae

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST - CYTE	2ND - CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Agallia sanguinolenta</i>		11♂	10, 11♂	10, 11♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
<i>Chlorotettix unicolor</i> ... } <i>Chlorotettix vividus</i> }	21 spg	11♂ 9♂	10, 11♂ 8, 9♂	10, 11♂ 8, 9♂	X to pole in 1st. Two species con- fused	Boring, '07	Jour. Exp. Zool., 4, p. 469
<i>Dicrocephala coccinea</i> . } <i>Dicrocephala mollipes</i> . }	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
<i>Phlepsius irroratus</i>	15 spg	8♂	7, 8♂	7, 8♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469

7. Membracidae

<i>Atymna castanea</i>		11♂	10, 11♂	10, 11♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
<i>Campylenchia curvata</i>	19 spg	10♂	9, 10♂	9, 10♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
<i>Ceresa bubalus</i> } <i>Ceresa diceros</i> } <i>Ceresa taurina</i> }		11♂	10, 11♂	10, 11♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
<i>Enchenopa binottata</i>	19 spg (20 in 2 cases)	10♂	10♂	9, 10♂	X to pole in 2nd	Boring, '07	Jour. Exp. Zool., 4, p. 469
<i>Enchenopa binottata</i>	20 spg 20 oog	10♂	10♂	10♂	XY to poles in 1st	Kornhauser, '14	Arch. Zellf., 12, p. 241 or Bull. Mus. Comp. Zool., Harvard, 244, p. 241
<i>Enchenopa curvata</i> (= <i>Campylenchia curvata</i>)	19 spg 20 oog	10♂	9, 10♂ (occasionally 19, no cell division)	9, 10♂	X to pole in 1st. Occasionally X divides	Kornhauser, '14	Arch. Zellf., 12, p. 241 or Bull. Mus. Comp. Zool., Harvard, 244, p. 241
<i>Entilia sinuata</i>	21 spg	11♂	10, 11♂	10, 11♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469

<i>Thelia bimaclata</i>	21 spg 22 oög	11♂			X to pole in 1st	Kornhauser, '14	Arch. Zellf., 12, p. 241 or Bull. Mus. Comp. Zool., Harvard, 244, p. 241
<i>Vanduzea arcuata</i>	17 spg	9♂	8, 9♂	8, 9♂	X to pole in 1st	Boring, '07	Jour. Exp. Zool., 4, p. 469
<i>b. Hymenoptera</i>							
<i>a. Apidae</i>							
<i>Apis mellifica</i> (= "Bienen").....	16 pa cl	16♀	8♀	8♀	Drone egg pa, with 2 p.b.s. Ootid chroms probably split in cl	Petrunkewitsch, '01 Petrunkewitsch, '03	Zool. Jahrb., 14, p. 573 Zool. Jahrb., 17, p. 481
<i>Apis mellifica</i>	16 spg	16♂	16♂	16♂	Half 1st spe rudimentary with no chroms. Half 2nd spe rudimentary with chroms	Möves, '03 Möves, '07	Anat. Anz., 24, p. 29 Arch. mikr. Anat., 70, p. 414
<i>Apis mellifica</i> (= "Honey-bee")		16♂	10♂	10♂	As above	Mark and Copeland, '06	Proc. Amer. Acad. Arts and Sci., 42, p. 101
<i>Apis mellifica</i>	16 oög	8♂ (double)	16♂	16♂	As above	Doncaster, '06 Doncaster, '07	Anat. Anz., 29, p. 490 Anat. Anz., 31, p. 168
<i>Apis mellifica</i>	16 spg (single) 16 oög (double) 16 pa cl 32 fert. cl	16♂ (dyads) 8♀ (dyads) 8♀ (dyads) 8♀ (dyads)	16♂ (dyads) 8♀ (dyads) 16♀ pron	16♂ (8 double) 8♀ (bivalent) 16♀ pron	As above	Nachtsheim, '12	Sitz. Gesel. Morph. u. Physiol. München, '12, p. 1 Arch. Zellf., 11, p. 169
<i>Osmia cornuta</i>	16 spg 32 or 64 som	16♂	16♂	8♂	Chroms pair at 1st anaphase, 1 mat. div. and half 1st spe rudimentary with chroms	Armbruster, '13 Armbruster, '13	Ber. Naturf. Ges. Freiburg, 20, p. 4 Arch. Zellf., 11, p. 242
<i>Xylocopa violacea</i>	16 spg Numerous in follicle cells	16♂	16♂	16♂	As above in Apis	Granata, '09 Granata, '13	Biologica 2, no. 15, p. 1 Mon. Zool. Ital., 24, p. 31
<i>b. Chalcididae</i>							
<i>Ageniaspis fuscicollis</i> (= <i>Eucyrtus</i>).....		ca 10♀			2 p.b.s in pa. Chroms fuse later	Silvestri, '08	Bol. R. Scuola Sup. Portici, 3, p. 29

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Agreniaspis fuscicollis</i>		4 ♀	4 ♀	4 ♀		Martin, '14	Zeit. wiss. Zool., 110, p. 419
<i>Copidosoma buyssoni</i>		12 ♀	10-12 ♀			Silvestri, '14	Anat. Anz., 47, p. 45
<i>Copidosoma gelechia</i>		12 ♀ Sometimes 11			May not be mat. spindle	Hegner, '14 Hegner, '15	Anat. Anz., 46, p. 51 Jour. Morph., 26, p. 495
<i>Prospalta berlesesi</i> (= <i>Prospaltella</i>).....		ca 10-12 ♀			1 p.b.	Silvestri, '08 Silvestri, '15	Boll. Lab. Zool. R. Sc., Agr. Portici, 3, p. 22 Boll. Lab. Zool. R. Sc., Agr. Portici, 10, p. 64
c. Cynipidae							
<i>Dryophanta erinacei</i>	12 som ♂ 13-14 som ♀		12 ♂		1st spc div. = cy- top. only	Wieman, '15	Biol. Bull., 28, p. 34
<i>Neotenus lenticularis</i> (= <i>Spathogaster bac- carum</i>).....	10 pa cl 10 som ♂ 20 pa cl 20 som ♀	10 ♀	10 ♀	10 ♀	Parthenogenetic. 2 types of eggs, one with p.b.s. may be ♀s; other lack- ing p.b.s. ♂s	Doncaster, '09 Doncaster, '11	Proc. Roy. Soc., 82B, p. 88 Proc. Roy. Soc. 83B, p. 476
	10 spg 20 oeg 18-20 cl 18-20 som	10 ♀	10 ♂	10 ♂ 10 ♀	Sexual. Half 1st spc rudimentary with no chroms		
	18 pa cl (= 18 -20)	9 ♀	9 ♀			Henking, '02	Zeit. wiss. Zool., 54, p. 1
<i>Rhodites rosae</i>	12 oeg 12 pa cl, later = 6 double	10-12 ♀ (prob. 12)	10-12 ♀	10-12 ♀		Schleip, '09	Zool. Anz., 35, p. 203
d. Formicidae							
<i>Formica sanguinea</i>	ca 24 pa cl ca 48 fert. cl	24 ♀	24 ♀	24 ♀	Pa and fert.	Schleip, '08	Zool. Jahrb., 26, p. 651
<i>Lasius niger</i>	16-17 pa cl 20 fert. cl	10 ♀	10 ♀			Henking, '92	Zeit. wiss. Zool., 54, p. 1

e. Tenthredinidae

<i>Crocus varus</i>		7-8 ♀, split later into 14 -16		Parthenogenetic	Doncaster, '06	Q. J. M. S., 49, p. 561
<i>Nematus lacteus</i>				Parthenogenetic	Doncaster, '06	Q. J. M. S., 49, p. 561
<i>Nematus ribesii</i>	ca 16 spg	ca 8 ♂ ca 8 ♀		1st spc div. abor- tive. Eggs par- thenogenetic. From correction in '09	Doncaster, '04 Doncaster, '06 Doncaster, '07 Doncaster, '08 Doncaster, '09 Doncaster, '10	Proc. Camb. Phil. Soc., 12, p. 474 Q. J. M. S., 49, p. 561 Q. J. M. S., 51, p. 101 Proc. Camb. Phil. Soc., 14, p. 22 Nature 82, p. 127 Science, 31, p. 192
<i>Poecilosoma luteolum</i>	8 pa cl	8 ♀ (possibly 7)		Parthenogenetic	Doncaster, '06 Doncaster, '06	Proc. Camb. Phil. Soc., 13, p. 103 Q. J. M. S., 49, p. 561

f. Vespidae

<i>Vespa crabro</i>		16+♀		1st spc rudimen- tary with no chroms	Meyes and Dues- berg, '08	Arch. mikr. Anat., 71, p. 571
<i>Vespa maculata</i>		16? ♂			Mark and Coppe- land, '07	Proc. Amor. Acad. Arts and Sci., 43, p. 69

G. *Lepidoptera*

a. Heterocera

1. Arctiidae

<i>Arctia caja</i>	31 ♂	31 ♂			Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
<i>Arctia hebe</i>	30-33 ♂				Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
<i>Chelonia caja</i>	24-28 ♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Hiporita jacobaea</i>	31 ♂				Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Phragmatobia fuliginosa</i> ...	prob. 56 spg 58♂ cl 58♀ cl Few 61, 62 cl	28♂ 28♀	28♂ 28, 29♀	28♂ 28, 29♀	XX in ♂ diploid. X + 3Y in ♀ di- ploid. XY in ♀ to poles in 1st. Y splits in two in 1st anaphase = 29	Seiler, '13 Seiler, '14	Zool. Anz., 41, p. 246 Arch. Zellf., 13, p. 159
<i>Spilosoma mendicum</i>		31♂	31♂			Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
2. Bombycidae							
<i>Bombyx mori</i>		12 ♀ at least				Henking, '92	Zeit. wiss. Zool., 54, p. 1
<i>Bombyx mori</i>	26-28 spg	28♂(26-28)	28♂	14♂	Half chroms. to each pole in 2nd	Toyama, '94 Toyama, '94	Zool. Anz., 17, p. 20 Imp. Univ. Tokyo Coll. Agr. Bull., vol. 11, no. 3, p. 125
<i>Bombyx mori</i> (17 varie- ties).....	50-60 spg 50-60 oog	28♂	28♂	28♂		Yatsu, '13	Annot. Zool. Japon- enses, 8, pt. 2, p. 215
<i>Theophilina naudiata</i> (= <i>Bombyx</i>).....		27♂	27♂			Yatsu, '13	Annot. Zool., Japon- enses, 8, pt. 2, p. 215
3. Geometridae							
<i>Abraxas grossulariata</i> <i>Abraxas leucolor</i> and cross	56 spg (50-56) 59 oog. 59 oog. in some strains	28♂(1 case 27) 28♀	28♂ or 26 + 1 pair in con- tact in 56 strains 27, 28♀ in 55 strains	28♂ 28♀ 27, 28♀	XX in ♂ diploid. X in ♀ X to pole in 2nd ♀	Doncaster, '10 Doncaster, '11 Doncaster, '12 Doncaster, '13 Doncaster, '14	Proc. Camb. Phil. Soc. 16, p. 44 Jour. Genetics, 1, p. 179 Jour. Genetics, 2, p. 189 Jour. Genetics, 3, pp. 1 and 29 Jour. Genetics, 4, p. 1
<i>Nyssia zonaria</i> (= <i>Ithy- sia</i>).....	ca 112 spg (100+)	56♂ 50-60♀ 31♂	56♂			Doncaster, '14	Jour. Genetics, 4, p. 1
<i>Ourapteryx sambucaria</i>						Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1

4. Lasiocampidae

<i>Cosmotriche potatoria</i>	31♂			Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
<i>Dendrolimus pini</i>	30♂	30♂		Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
<i>Malacosoma castrense</i>	31♂			Kernewitz, '15	Arch. Naturges., 81, p. 1

5. Lymantriidae

<i>Leucoma salicis</i>	12+♀	31♂	31♂	Henking, '92	Zeit. wiss. Zool., 54, p. 1
<i>Lymantria dispar</i> } <i>Lymantria japonica</i> }	62 cl	31♂ 31♀		Seiler, '14	Arch. Zellf., 13, p. 159
<i>Orygia antiqua</i>	14♀			Seiler, '14	Arch. Zellf., 13, p. 159
<i>Orygia gonostigma</i>	30♀			Seiler, '14	Arch. Zellf., 13, p. 159

6. Monoctenidae

<i>Lycia hirtaria</i> (= Biston)	28 spg 28 oog	13♂ (1 double) 13 + chrom. nucleolus ♀	13 (1 double), 14♂	Possibly X to pole in 1st	Jour. Genetics, 3, p. 229
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7. Noctuidae

<i>Acronycta</i> sp?.....		29♂		Cook, '10	Proc. Acad. Nat. Sci. Phila., 62, p. 294
<i>Agrostis triangulum</i>		29♂	29♂	Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
<i>Leucania impura</i>		31♂	31♂	Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1

8. Notodontidae

<i>Dicranura vinula</i>		21♂		Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
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II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Phaleria bucephala</i>	ca 60 spg	30♂ (some- times 31)	30♂			Kernowitz, '15	Arch. Naturges., 81, p. 1
<i>Pygarea anchorata</i>		30♂	12-14♂		Different in apy- rene	Federley, '13	Zeit. ind. Abs. u. Vererb., 9, p. 1
<i>Pygarea bucephala</i>		29♂	29♂			Platner, '86	Intern. Monats. Anat. u. Physiol., 3, p. 341
<i>Pygarea curtula</i>		23♂	23♂			Federley, '13	Zeit. ind. Abs. u. Vererb., 9, p. 1
<i>Pygarea pigra</i>	40+ spg				Different in apy- rene	Federley, '13	Zeit. Ind. Abs. u. Vererb., 9, p. 1
9. Pyralidae							
<i>Ephestia kuehniella</i>		29♂	29♂			Kernowitz, '15	Arch. Naturges., 81, p. 1
<i>Galleria melonella</i>	ca 60 spg	30♂			Different in apy- rene	Von Kennitz, '14	Arch. Zelf., 12, p. 567
10. Saturniidae							
<i>Antheraea pernyi</i>		31♂	33♂ (some- times 34)			Kernowitz, '14 Kernowitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
<i>Automeris io</i>		19♂	19♂	19♂	Equal XY	Cook, '10	Proc. Acad. Nat. Sci., Phila., 64, p. 294
<i>Callosamia promethea</i> ...	38 spg	13♂ 13 ♀ 26 som	13♂ 13 ♀ 26 som	13♂ 13 ♀	Equal XY	Cook, '10	Proc. Acad. Nat. Sci., Phila., 64, p. 294
<i>Philosamia cyathia</i>	26 spg 26 oog 26 som	13♂ 13 ♀ 26 som	13♂ 13 ♀ 26 som	13♂ 13 ♀		Dederer, '07 Dederer, '15	Biol. Bull., 13, p. 94 Jour. Morph., 26, p. 1
<i>Philosamia cyathia</i>	26 spg	13♂	13♂			Cook, '10	Proc. Acad. Nat. Sci., Phila., 64, p. 294
<i>Samia cecropia</i>		30♂	30♂		Equal XY	Cook, '10	Proc. Acad. Nat. Sci., Phila., 64, p. 294

<i>Saturnia pavonia</i>	29♂	29♂	Kernewitz, '15	Arch. Naturges., 81, p. 1
<i>Telega polyphemus</i>	30♂	30♂	Cook, '10	Proc. Acad. Nat. Sci. Phila., 64, p. 234
11. Spingidae				
<i>Cherocampa elpenor</i>	29♂	29♂	Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
<i>Deilephila euphorbiae</i>	28-29♂		Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
<i>Deilephila euphorbiae</i>	28 spg	28♂ (some- times 29)	Bader, '15	Arch. Zellf., 14, p. 26
<i>Dilina tiliae</i>	29♂	29♂	Federley, '14	Öfver. Finsk. Vetensk. Soc. Förhand., 56, p. 1
<i>Dilina tiliae</i>	29♂	29♂	Kernewitz, '15	Arch. Naturges., 81, p. 1
<i>Smerinthus ocellatus</i>	27♂	27♂	Federley, '14	Öfver. Finsk. Vetensk. Soc. Förhand., 56, p. 1
<i>Smerinthus ocellatus</i>	28♂	28♂	Kernewitz, '15	Arch. Naturges., 81, p. 1
<i>Smerinthus populi</i>	28♂	28♂	Federley, '14	Öfver. Finsk. Vetensk. Soc. Förhand., 56, p. 1
<i>Sphinx ligustri</i>	27-29♂	27-29♂	Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
12. Tortricidae				
<i>Cacaecia cerasivorana</i>	30♂	30♂	Stevens, '06	Carnegie Inst. Pub., 36, II, p. 33

II. ARTHROPODA—Continued
 b. Rhopalocera
 1. Nymphalidae

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Euanassa antiopa</i>		30♂			Equal XY	Stevens, '06	Carnegie Inst. Pub., 36, 11, p. 33
<i>Polygonia-c-album</i>			31♂			Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
2. Papilionidae							
<i>Papilio podalirius</i>	54-58 spg					Kernewitz, '15	Arch. Naturges., 81, p. 1
<i>Papilio rutulus</i>	28 spg	28♂	28♂	14♂		Munson, '06	Proc. Boston Soc. Nat. Hist., 33, p. 43
3. Pieridae							
<i>Aporia crataegi</i>			25♂			Kernewitz, '14 Kernewitz, '15	Zool. Anz., 45, p. 137 Arch. Naturges., 81, p. 1
<i>Colias myrmidone</i>			30-31♂			Kernewitz, '15	Arch. Naturges., 81, p. 1
<i>Pieris brassicae</i>	ca 30 spg ca 28 oog ca 25 cl	14 or 15♂ 14 ♀	14 ♀	14 ♀		Henking, '90 Henking, '90 Henking, '91 Henking, '92	Intern. Monats. Anat. u. Physiol., 7, p. 243 Zets. wiss. Zool., 49, p. 303 Zets. wiss. Zool., 51, p. 685 Zets. wiss. Zool., 54, p. 1
<i>Pieris brassicae</i>	ca 30 spg 30 oog	15♂ (= 14 + chromatin nucleolus) One cell 25	15♂		Possibly XY in ♂ No XY in ♀	Doncaster, '12 Doncaster, '12	Proc. Camb. Phil. Soc., 16, p. 491 Jour. Genetics, 2, p. 189
<i>Pieris napi</i>	50? spg	25♂				Henking, '90	Intern. Monats. Anat. u. Physiol., 7, p. 243

7. *Neuroptera*a. *Corrodentia*

<i>Cerastipsocus venosus</i>	17 spg	9♂	8, 9♂	8, 9♂	X to pole in 1st	Boring, '13	Biol. Bull., 24, p. 125
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b. *Isoptera*

<i>Termopsis angusticollis</i> ...	52 spg	26♂	26♂	No X		Stevens, '05	Carnegie Inst. Pub., 36, p. 1
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c. *Odontata*

<i>Anax junius</i>	27 spg 28 follicle cells	14♂	14♂	13, 14♂	X to pole in 2nd. from correction in '08	McGill, '04 Lefevre and Mc- Gill, '08	Univ. Missouri Studies, 2, no. 3, p. 1 Amer. Jour. Anat., 7, p. 469
<i>Calopteryx virgo</i>		8-14♂	5-7♂			Carnoy, '85	La Cellule, 1, p. 189

d. *Trichoptera*

<i>Panorpa communis</i>		14-18♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Platyphax designatus</i>	55-60 oog	30♂	30♂		Chromatin nucleus divides in both divisions	Lutman, '10	Biol. Bull., 19, p. 55

8. *Orthoptera*a. *Acerididae*

<i>Aceridium granulatus</i> see under <i>Tettigidae</i>		10-16♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Aceridium linolea</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Acerolophitus</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Aeoloplus</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Amphitornus</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Arphia</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Arphia pseudonietana</i>	23 spg					Meek, '13	Phil. Trans. Roy. Soc., London, 203B, p. 1.

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Arphia simplex</i>	23 spg	12♂			X to pole in 1st	Carothers, '13	Jour. Morph., 24, p. 487
<i>Arphia tenebrosa</i>	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st. One animal had 2 X, to same or op- posite poles in 1st; = 24 spg, 13 1st, 11, 12, 13 2nd	Davis, '08	Bull. Mus. Comp. Zool. Harvard, 53, p. 57
<i>Aukocera</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Boopeton</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Brachystola</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Brachystola magna</i>	23 spg 22 oog	12♂	11, 12♂	11, 12♂	X to pole in 1st	Sutton, '02	Biol. Bull., 4, p. 24
<i>Brachystola magna</i>	23 spg	12♂		11, 12♂	X to pole in 1st	Carothers, '13	Jour. Morph., 24, p. 487
<i>Culeptenus femur-rub- rum</i> , see <i>Melanoplus</i> (<i>Amnula</i>).....	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Chortophaga viridifasci- ata</i>	19 spg (= 23) or 23 spg	12♂	11, 12♂		X to pole in 1st. Union of chroms. in spg to form te- trads	McClung, '05 McClung, '14	Biol. Bull., 9, p. 304 Jour. Morph., 25, p. 651
<i>Chortophaga viridifasci- ata</i>	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Davis, '08	Bull. Mus. Comp. Zool. Harvard, 53, p. 57
<i>Climocephalus</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Dactyotolum</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651

Disostetra.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
Disostetra carolina.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Davis, '08	Bull. Mus. Comp. Zool. Harvard, 53, p. 57
Encoptolophus.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
Eremnus.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
Hadrotetrix.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
Hesperotetrix pratensis Hesperotetrix spectosus Hesperotetrix viridis }	22 spg (= 23)	11♂ (= 12)			X attached to another chromosome in spg and 1st. To pole in 1st	McClung, '05 McClung, '14	Biol. Bull., 9, p. 304 Jour. Morph., 25, p. 651
Hippiscus phoenicopterus.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
Hippiscus tuberculatus.....	23 spg 24 oög	12♂	11, 12♂	11, 12♂	X to pole in 1st	Davis, '08	Bull. Mus. Comp. Zool. Harvard, 53, p. 57
Mecostethus.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
Melanoplus.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
Melanoplus angustipennis.....	23 spg		11, 12♂	11, 12♂	X	Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
Melanoplus atlantis.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Nowlin, '12	Kansas Univ. Sci. Bull., 6, p. 399
Melanoplus atlantis.....	23 spg		11, 12♂	11, 12♂	X	Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
Melanoplus bivittatus.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Nowlin, '08	Kansas Univ. Sci. Bull., 4, p. 205
Melanoplus bivittatus.....	23 spg					Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
Melanoplus dawsonii.....	23 spg					Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Melanoplus differentialis</i> ...	23 spg	12♂	11, 12♂		X to pole in 1st	Nowlin, '12	Kansas Univ. Sci. Bull., 6, p. 399
<i>Melanoplus femoratus</i> ...	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Davis, '08	Bull. Mus. Comp. Zool. Harvard, 53, p. 37
<i>Melanoplus femur-rubrum</i> (= <i>Chloptenus</i>)...	12 spg 12 som	6♂ (= 24 el)	6♂ (= 12 el)	6♂	Tetrads and dyads separate ele- ments	Wilcox, '04 Wilcox, '05	Anat. Anz., 10, p. 303. Bull. Mus. Comp. Zool. Harvard, 27, p. 3.
<i>Melanoplus femur-rubrum</i>	23 spg 22? oog	12♂	11, 12♂	11, 12♂	X to pole in 1st	Wilcox, '08	Anat. Anz., 14, p. 194
<i>Melanoplus packardii</i>	23 spg	12♂	11, 12♂		X to pole in 1st	Nowlin, '12	Kansas Univ. Sci. Bull., 6, p. 399
<i>Melanoplus packardii</i>	23 spg	12♂	11, 12♂		X to pole in 1st	Nowlin, '12	Kansas Univ. Sci. Bull., 6, p. 399
<i>Merniria bivittata</i>	23 spg	10♂ (= 12)	11, 12♂		X	Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
	21 spg (= 23)				X attached to two other chromo- somes, one of which passes to each pole in 1st, X going to pole with one of them	McClung, '05 McClung, '14	Biol. Bull., 9, p. 304 Jour. Morph., 25, p. 651
<i>Mestobregma</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Oedipoda</i>	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Buchner, '09	Arch. Zellf., 3, p. 335
<i>Orphulella</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Pamphagus marmoratus</i> ...	19 spg 20 ♀ som	10♂			X to pole in 1st	Granata, '10	Arch. Zellf., 5, p. 182
<i>Paratettix</i> , see under Tettigidae							
<i>Paroxya</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651

<i>Philobostroma</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Phoetaliotes</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Phrynotettix</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Phrynotettix magnus</i>	23 spg	12♂	11, 12♂	X to pole in 1st	Pinney, '08	Kansas Univ. Sci. Bull., 4, p. 309
<i>Prorocorypha</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Pseudopomula</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Psimidia</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Rhomaleum</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Selisticocera</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Selisticocera alutacea</i> }	23 spg	12♂			Hartmann, '13	Biol. Bull., 24, pp. 226 and 239
<i>Selisticocera americana</i> }	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Scirtettica</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Spharagemon</i>	23 spg	12♂	11, 12♂	X to pole in 1st	Artom, '09	Biologica, 2, no. 16, p. 1
<i>Stauronotus maroccanus</i>	23 spg	12♂	11, 12♂	X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Stenobothrus</i> (prob. = <i>Chorhippus</i>).....	21 spg	11♂	10, 11♂	X to pole in 1st	Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Stenobothrus bicolor</i>	17 spg	9♂	8, 9♂	X to pole in 1st	Gérard, '09	Bull. Soc. Roy. Sci. med. et nat. Bruxelles, 67, p. 23
<i>Stenobothrus biguttulus</i>	16-17 spg (prob. 17)	9♂	8, 9♂	X to pole in 1st	Gérard, '09	Arch. Biol., 24, p. 543
<i>Stenobothrus curtipes</i>	17 spg	9♂	8, 9♂	X to pole in 1st	Davis, '08	Bull. Mus. Comp. Zool. Harvard, 53, p. 57

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARHENOGENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Stenobothrus curtipes</i>	17 spg	9♂	8, 9♂			Meek, '12	Jour. Linnean Soc., 32, p. 107
<i>Stenobothrus parallelus</i>	17 spg		8, 9♂		X to pole in 1st	Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Stenobothrus viridulus</i> (or bicolor).....		12-18♂	6-8♂			Carnoy, '85	La Cellule, 1, p. 189
<i>Stenobothrus viridulus</i>	17 spg	9♂	8, 9♂		X to pole in 1st	Meek, '11	Jour. Linnean Soc., 32, p. 1
						Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Syrbula</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Syrbula acuticornis</i>	29 spg	10♂	10♂	10♂	Bivalent heterochrom, divides in both divisions	Montgomery, '05	Proc. Acad. Nat. Sci. Phila., 57, p. 162
<i>Syrbula admirabilis</i>	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Robertson, '08	Kansas Univ. Sci. Bull., 4, p. 275
Tettigidae (subfamily of Acrididae). <i>Acridium granulatus</i> . <i>Paratettix</i> <i>Tettigidea parvipennis</i> }	13 spg 14 oog	7♂			X to pole in 1st	Robertson, '08	Kansas Univ. Sci. Bull., 4, p. 275
						Robertson, '15	Jour. Morph., 26, p. 109
<i>Paratettix leuconotus-leucothorax</i>	13 spg	7♂	6, 7♂		X to pole in 1st	Harman, '15	Biol. Bull., 24, p. 262
<i>Trimerotropis</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Tropidolophus</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651
<i>Tryxalis</i>	23 spg	12♂	11, 12♂		X to pole in 1st	McClung, '14	Jour. Morph., 25, p. 651

Tryxalis nasuta.....	21 spg	11♂		X to pole in 1st	Brunelli, '10 Brunelli, '11	Mem. Soc. ital. Sci., ser. 3a, vol. 16, p. 221 Mem. R. Acad. Lin- cea, ser. Va, vol. 8, p. 633
b. Blattidae						
Blatta germanica.....		12♂			Erlanger, '96 Erlanger, '97	Zool. Anz., 19, p. 65 Zool. Centralb., 4, p. 1
Blatta germanica.....	23 spg 24 oog	12♂	11, 12♂	X to pole in 1st	Wassilieff, '07	Arch. mikr. Anat., 70, p. 1
Blatella germanica (= Blatta).....	23 spg 237 ♀ som	12♂	11, 12♂	X to pole in 1st	Stevens, '05	Carnegie Inst. Pub. no. 36, p. 1
Leucophaea maderiae.....	23 spg 24 oog	11, 12♂	11, 12♂	X to pole in 1st	Morse, '09	Arch. Zellf., 3, p. 483
Periplaneta americana.....	32 spg	16♂ (gemini)	16♂		Farmer and Moore, '04 Moore and Rob- inson, '04 Moore and Ar- nold, '05	Q. J. M. S., 48, p. 489 Q. J. M. S., 48, p. 571 Proc. Roy. Soc., 77, p. 563
Periplaneta americana.....	33 spg 34 oog	17♂	16, 17♂	X to pole in 1st	Morse, '09	Arch. Zellf., 3, p. 483
c. Forficulidae						
Anisolabis maritima.....	24 spg 24 oog 24 som	12♂	12♂	Equal pair hetero- chromosomes	Randolf, '08	Biol. Bull., 15, p. 111
Forficula auricularia.....		10-14♂			Carney, '85	La Cellule, 1, p. 189
Forficula auricularia.....		12♂	12-14♂		St. George, '87	Festschrift. f. Kölli- ker, p. 51
Forficula auricularia.....	24 spg	12♂	12♂		Sinécy, '01	La Cellule, 19, p. 117
Forficula auricularia.....	24 or 26 spg	12-14♂	12-14♂	One accessory chrom. (13) or two (14) or none (12)	Zweiger, '06 Zweiger, '06	Zool. Anz., 30, p. 220 Jen. Zeits., 35, p. 143
Forficula auricularia.....	24 spg	12♂	12♂ (occa- sionally 11 and 13)	XY to poles in 1st	Stevens, '10	Jour. Exp. Zool., 8, p. 227

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Forficula auricularia</i>	24 spg	12♂	12♂		Pair heterochromosomes in 2nd	Meek, '13 Meek, '13 Meek, '15	Phil. Trans. Roy. Soc., London, 203B, p. 1 Q. J. M. S., 59, p. 249 Q. J. M. S., 61, p. 1
<i>Forficula auricularia</i> (?)...	24-27 spg	12-14♂	11-14 ♀	10-14♂	Some chroms. uni-valent in 1st and may or may not divide	Payne, '14	Jour. Morph., 25, p. 559
<i>Labidura riparia</i>		6♂				Sinétý, '01	La Cellule, 19, p. 117
d. Gryllidae							
<i>Gryllotalpa</i>	23 spg	12♂				Baumgartner, '12	Science, 35, p. 937. (Paper read before Amer. Soc. Zool.)
<i>Gryllotalpa borealis</i>	23 spg 24 oog	12♂	11, 12♂		X and XY to poles in 1st	Payne, '12	Arch. Zellf., 9, p. 141
<i>Gryllotalpa vulgaris</i>	12 spg	6♂ (= 24 el)	6♂ (= 12 el)	6♂	Tetrads and dyads = separate elements	Vom Rath, '92 Vom Rath, '95	Arch. mikr. Anat., 40, p. 102 Arch. mikr. Anat., 46, p. 168
<i>Gryllotalpa vulgaris</i>	17 spg	9♂			X to pole in 1st	Senna, '11	Mon. Zool. ital., 22, p. 65
<i>Gryllotalpa vulgaris</i>	16 spg (= 17, one bivalent)	7♂	7♂	7♂	XY to poles in 1st. X united with a tetrad. X to pole in 1st. Two tetrads united in 1st. From correction in '14	Voinov, '12 Voinov, '14	C. R. Assoc. Biol., 72, p. 621 Arch. Zool. Exper. et gen., 54, p. 439
<i>Gryllus assimilis</i> (actuosus?).....	29 spg	15♂	14, 15♂		X to pole in 1st	Baumgartner, '04	Biol. Bull., 8, p. 1
<i>Gryllus desertus</i>	21 spg	11♂			X to pole in 1st	Brunelli, '09	Mem. R. Acad. Lincei., ser. 5a., vol 7, p. 623
<i>Gryllus domesticus</i>	21 spg	11♂	10, 11♂	10, 11♂	X to pole in 1st	Baumgartner, '04	Biol. Bull., 8, p. 1

Gryllus domesticus.....	21 spg 22 oög 21♂ som 22 ♀ som	11♂	11♂	10, 11♂	X in ♂; XX in ♀	Guthertz, '07 Guthertz, '08 Guthertz, '09 Meek, '13	Arch. Mikr. Anat., 69, p. 491 Zentr. Physiol., 22, p. 61 Gesel. Naturf. Fr. Berlin, 1909, pp. 410 and 565 Phil. Trans. Roy. Soc., London, 203B, p. 1
Gryllus domesticus.....	21 spg	11♂	11♂	10, 11♂	X to pole in 1st		
e. Locustidae							
Anabrus.....	33 spg	17♂	16, 17♂	16, 17♂	X to pole in 1st	McClung, '02 McClung, '14	Kansas Univ. Sci. Bull., 1, p. 185 Jour. Morph., 25, p. 651
Ceuthophilus sp?.....	37? spg	19♂ or 21♂ (2S)	11, 12♂	11, 12♂	X to pole in 1st, 2S to either pole in 1st or 2nd	Stevens, '12 McClung, '14	Biol. Bull., 22, p. 219 Jour. Morph., 25, p. 651
Conocephalus.....	33 spg						
Decticus verrucivorus.....	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Vejdovsky, '12	Kgl. Böhm. Gesel. Wissen. Prag, 1912, p. 1
Decticus verrucosus.....	31 spg					Buchner, '09	Arch. Zellf., 3, p. 335
Diastrammena marmorata.....	57 spg	28♂	28, 29♂	28, 29♂	X to pole in 1st	Schellenberg, '13	Arch. Zellf., 11, p. 489
Jamaicana flava Jamaicana subguttata Jamaicana unicolor	35 spg	18♂	17, 18♂	17, 18♂	X to pole in 1st, Association of 2 chroms. in some individuals	Woolsey, '15	Biol. Bull., 28, p. 163
Leptophyes punctatissima.....	31 spg 32 oög 31♂ som 32 ♀ som				X to pole in 1st, XX in ♀ diploid	Mohr, '15	Arch. Zellf., 14, p. 151
Locusta viridissima.....	33 spg	17♂	16, 17♂	16, 17♂	X to pole in 1st	Otte, '06 Otte, '07	Zool. Anz., 30, p. 529 Zool. Jahrb., 24, p. 431
Locusta viridissima.....	29 spg 30 oög 29♂ som 30 ♀ som				X to pole in 1st, XX in ♀ diploid	Mohr, '15	Arch. Zellf., 14, p. 151

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Microcentrum</i>	32? spg	17♂	16, 17♂	16, 17♂	X to pole in 1st	McClung, '02	Kansas Univ. Sci. Bull., 1, p. 185
<i>Orchesticus</i>	33 spg	17♂	16, 17♂	16, 17♂	X to pole in 1st	McClung, '02 McClung, '14	Kansas Univ. Sci. Bull., 1, p. 185 Jour. Morph., 25, p. 651
<i>Orphanina denticulata</i>	31 spg	15♂ (= 16, X at pole in metaphase)	15, 16♂	15, 16♂	X to pole in 1st	Sinétý, '01	La Cellule, 19, p. 117
<i>Scudderia</i>	33 spg	17♂	16, 17♂	16, 17♂	X to pole in 1st	McClung, '02 McClung, '14	Kansas Univ. Sci. Bull., 1, p. 185 Jour. Morph., 25, p. 651
<i>Steiroxys trilineata</i>	29 spg	15♂	14, 15♂	14, 15♂	X to pole in 1st	Davis, '08	Bull. Mus. Comp. Zool. Harvard, 53, p. 57
<i>Steiroxys trilineata</i>	29 spg	15♂	14, 15♂	14, 15♂	X to pole in 1st	Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Stenopelmatus</i>	47 spg		23, 24♂		X to pole in 1st. From correction in '09	Stevens, '05 Stevens, '09	Carnegie Inst. Pub. no. 36, p. 1 Jour. Exp. Zool., 6, p. 101
<i>Troglophilus</i> sp?.....	ca. 20 oog					Buehner, '10	Arch. Zellf., 5, p. 449
<i>Xiphidium</i>	33 spg	17♂	16, 17♂	16, 17♂	X to pole in 1st	McClung, '02 McClung, '08 McClung, '14	Kansas Univ. Sci. Bull., 1, p. 185 Kansas Univ. Sci. Bull., 4, p. 255 Jour. Morph., 25, p. 651
f. Mantidae							
<i>Mantis religiosa</i>	14 spg	14♂ (double = 28)	7♂ (double = 14) 14♀	7♂, 14♂ pron 7♀, 14♀ pron	Chroms. divide just before fertiliza- tion	Giardina, '97	Mon. Zool. ital., 8, p. 275

g. Phasmodae

<i>Aplopus mayeri</i>	35 spg. 36 ♀ som	18♂	17, 18♂	17, 18♂	X to pole in 1st	Jordan, '08 Jordan, '08	Anat. Anz., 32, p. 284 Carnegie Inst. Pub. 102, p. 13
<i>Bacillus linearis</i>		8-10♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Bacillus rossii</i>	20 oog 20 cl	18-20 ♀	ca. 20 ♀		Parthenogenetic. polar bodies	von Baehr, '07 La Cellule, '12	Zool. Jahrb., 24, p. 175 La Cellule, 27, p. 383
<i>Leptynia attenuata</i>	36 spg (= 37) 36 oog	18♂ (= 19)	18♂ (= 18, 19)		X attached to an- other chrom. To pole in 1st	Sinety, '01	La Cellule, 19, p. 117

d. MYRIAPODA

1. Chilopoda

<i>Geophilus linearis</i>		8♂	8♂	8♂		Bouin and Collin, '01 P. and M. Bouin, '03	Anat. Anz., 20, p. 97 C. R. Soc. Biol., 55, p. p. 763
<i>Lithobius forficatus</i>		16-24♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Lithobius forficatus</i>		22-24♂				P. and M. Bouin, '02	C. R. Assoc. Anat., 4, p. 74
<i>Lithobius mordax</i> <i>Lithobius multidentatus</i> <i>Lithobius</i> sp?		25♂			X	Blackman, '07	Proc. Amer. Acad. Arts and Sci., 42, p. 487
<i>Scolopendra dalmatica</i>		20-24♂				Carnoy, '85	La Cellule, 1, p. 189
<i>Scolopendra heros</i>	33 spg	17♂	16, 17♂	16, 17♂	X to pole in 1st	Blackman, '03 Blackman, '03	Biol. Bull., 5, p. 187 Bull. Mus. Comp. Zool. Harvard, 48, p. 1
<i>Scutigera coleoptrata</i>		18♂			Accessory divides in both divisions	Blackman, '10 Bouin and Ancel, '11	Biol. Bull., 19, p. 138 C. R. Assoc. Anat., 13, p. 104
<i>Scutigera forceps</i>	37 spg	19♂	18, 19♂		X to pole in 1st	Medes, '05	Biol. Bull., 9, p. 156

2. Diplopoda

<i>Pachyiulus varius</i>	25 spg	13♂	12, 13♂		X to pole in 1st	Oettinger, '08 Oettinger, '09	Zool. Anz., 33, p. 164 Arch. Zellf., 3, p. 563
<i>Polyxenus</i> sp?.....	prob. 16 spg	8♂	8♂			Sokoloff, '14	Zool. Anz., 44, p. 558

II. ARTHROPODA—Continued
c. ONYCHOPHORA

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Peripatus.....	28 spg (23-34) 28 oog 28 som	14♂	14♂	14♂		Montgomery, '00	Zool. Jahrb., 14, p. 277

III. COELENTERATA

a. HYDROZOA
1. Leptothecae
a. Anthomedusae

Clava squamata.....		ca. 16 ♀				Harm, '02	Zeit. wiss. Zool., 73, p. 115
Clavarella prolifera (= Eleutheria dichotoma)		6 ♀				Muller, '08	Zeit. wiss. Zool., 89, p. 28
Cordylophora lacustris...		10-12 ♀				Morgenstein, '01	Zeit. wiss. Zool., 70, p. 367
Eudendrium ramosum...		13? ♀				Beckwith, '14	Jour. Morph., 25, p. 189
Hydra dioecia } Hydra fusca } Hydra viridis }	12 spg	6♂ 12 ♀	6♂ 6 ♀	6♂ 6 ♀	Reduction in 1st anaphase in ♀	Downing, '05 Downing, '09	Zool. Jahrb., 21, p. 379 Zool. Jahrb., 28, p. 293
Hydra grisea.....		12-14 ♀				Brauer, '91	Zeit. wiss. Zool., 52, p. 169
Hydra grisea.....		16 ♀				Wager, '09	Biol. Bull., 18, p. 1
Hydractinia echinata.....	12-16 cl					Smallwood, '09	Biol. Bull., 17, p. 209
Hydractinia echinata.....		14? ♀				Beckwith, '14	Jour. Morph., 25, p. 189
Pennaria tiarella.....		10-14 ♀ (prob. 10)				Hargitt, '09	Bull. Mus. Comp. Zool. Harvard, 53, p. 161
Pennaria tiarella.....	14? cl					Smallwood, '09	Biol. Bull., 17, p. 209

Tiara sp?.....	28 cl	14 ♀	14 ♀	14 ♀	Boveri, '90	Zellenstudien III, or Jen. Zeits., 17, p. 314
Tubularia mesembryan- themum.....		ca. 12 ♀			Brauer, '91	Zeit. wiss. Zool., 52, p. 351
b. Leptomedusae						
Aequorea forskalea.....	12 cl		6 ♀		Häcker, '92	Arch. mikr. Anat., 40, 243
Campanularia fluxuosa...	20 cl	10 ♀			Hargitt, '13	Jour. Morph., 24, p. 383
Gonothyrea loveni.....		8 ♀			Wulfert, '02	Zeit. wiss. Zool., 71, p. 206
2. Trachylinae						
Cunina proboscidea.....		28+♂ 30 ♀		14+♂	Stschelkanowzew, '06	Mit. Zool. St. Neapel, 17, p. 433
Gonionemus murbachii...	24-25 spg prob. 24 oog 24 cl prob. 24 som	prob. 12♂	prob. 12♂	prob. 12 ♀	Bigelow, '07	Bull. Mus. Comp. Zool. Harvard, 48, p. 287
b. Scyphozoa						
Aurelia flavidula.....	18-20 cl	9-10 ♀			Hargitt, '10	Jour. Morph., 21, p. 217

EXPERIMENTAL CONTROL AND MODIFICATION OF LARVAL DEVELOPMENT IN THE SEA URCHIN IN RELATION TO THE AXIAL GRADIENTS

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EIGHT PLATES

The literature of experimental teratogeny in embryonic and larval development is extensive and a great variety of external factors has been employed in experimentation along this line. While the results have often been of great interest, no general basis for interpretation has been reached, particularly as regards the relation between teratological form and the action of experimental factors to which the developing organism is subjected as a whole, and not by local application. It is a familiar fact that even such factors affect different regions or parts differently or to a different degree, and while such differences indicate the existence of local differences of some sort, there is, in general, a very evident lack of specificity in the action of different external factors on the course of organic development. Similar teratological forms may often be produced by many different agents and conditions.

The present paper constitutes one step in the attempt to correlate certain types of teratological development with dynamic conditions which are characteristic and fundamental features of the normal organism. It establishes a basis for the control, modification and prediction of the course of development in the sea urchin, and the facts already at hand indicate very clearly that certain types of teratological development, as well as certain characteristic features of normal development, can readily be interpreted on the same basis. More specifically this paper is a demonstration of the effectiveness of the axial metabolic gradients as dynamic factors in the development of the sea

urchin. It shows how, and to what extent, it is possible to control and modify development by means of the differential action of external factors on different regions of these gradients.

THE NATURE AND PURPOSE OF THE EXPERIMENTS

The existence of an axial metabolic gradient, at least in the major or polar axis with the apical region as the region of highest rate of reaction, or, perhaps, as possessing the capacity for the highest rate of reaction has been demonstrated in many species of animals and plants (Child, '13 b, '14 a, '15 a, '15 c, Chap. III, '16 a, '16 c; Hyman, '16), including the developmental stages of the sea urchin. This demonstration has been made possible by the differences in susceptibility to various agents which are correlated with the differences in metabolic rate and protoplasmic condition at different levels of the gradient.

So far as this relation between susceptibility to inhibiting agents and metabolic rate has been investigated, it is as follows. In concentrations of cyanides, narcotics so far as tested, acids, and, under certain conditions, alkalis, which kill, not immediately but rapidly enough so that the organism does not become acclimated or acquire a tolerance to them, the susceptibility varies directly with the general metabolic rate or with the rate of certain fundamental metabolic reactions. To low concentrations, to which more or less acclimation is possible, the degree and rapidity of acclimation vary in general directly with the metabolic rate—with certain exceptions (Child, '14 b)—and within certain limits of low concentration the parts least acclimated die, while those more fully acclimated remain alive, so that in the long run the susceptibility varies inversely as the metabolic rate (Child, '13 a, '15 b, Chap. III).

In *Planaria*, for example, it has been shown (Child, '13 b) that to the higher concentrations of cyanides and narcotics the susceptibility decreases from the anterior end posteriorly, while in the very low concentrations the degree of acclimation is greatest at the anterior end (Child, '12), and in the long run susceptibility is greatest at the posterior end and decreases anteriorly.

From the metabolic point of view, acclimation consists in the gradual increase in rate of reaction in the presence of the inhibiting agent, and the inverse relation between capacity for acclimation and metabolic rate means that, the higher the metabolic rate, the greater the capacity for gradual increase of rate after the first inhibiting action.

Recovery after the temporary action of an inhibiting agent consists, like acclimation, in the attainment of a higher metabolic rate, but the rate attained is much higher than in acclimation because the inhibiting agent is completely removed. Where the action of the inhibiting agent has not gone so far that recovery is impossible, the rapidity and degree of recovery like acclimation vary directly with metabolic rate; the higher the metabolic rate the more rapid and complete the recovery.

If these conclusions are correct and if axial gradients are present in organisms and are actual effective factors in development, these relations between susceptibility and metabolic rate afford a basis for the control and modification of development in two opposite directions. First, by the use of concentrations of an inhibiting agent sufficiently high to prevent acclimation to the reagent, or concentrations and periods of action sufficient to prevent recovery after temporary subjection, it should be possible to inhibit the regions of higher rate of reaction in an axial gradient to a greater extent than those of lower rate. If the rate of reaction in the apico-basal axial gradient decreases from the apical region basally, the apical region should be most inhibited, the basal least under such conditions. Second, by the use of low concentrations which permit some degree of acclimation, or by temporary action of concentrations whose effect is readily reversible, it should be possible to obtain a greater degree of acclimation or more rapid and complete recovery in the region of high rate, i.e., the apical region, and so to inhibit its development to a lesser extent than that of the regions of lower rate. In short, it should be possible to produce, in the one case, a gradient or gradients in inhibition of development, and, in the other, gradients in acclimation or recovery. In fact, by determining proper concentrations and times of exposure, it

should be possible, not only to produce differential or graded inhibition or acceleration of development along an axis, but to determine the position of the greatest degree of inhibition or acceleration, either at the high or low end, the region of highest or that of lowest rate in the axis. To obtain a particular degree of differential inhibition, the procedure must of course be varied somewhat according to the toxicity of the reagent.

The experiments on the developmental stages of the sea urchin were undertaken with these ideas in mind and with the purpose of demonstrating that the axial gradients are determining and effective factors in embryonic development. The results of the experiments have completely realized expectation and leave no doubt as to the fundamental character of the rôle played by the axial gradients in the development of the sea urchin. Some similar data on the starfish, which I hope to supplement by further experiments before publication, indicate that the relations are essentially the same there as in the sea urchin.

THE AXIAL RELATIONS IN NORMAL DEVELOPMENT

Throughout the paper the terms 'apical' and 'basal' are used to designate the two ends of the apico-basal, polar, or major axis, the apical end representing the apical or 'animal,' the basal end the 'vegetative' pole of the egg. In speaking of metabolic gradients, the ends or levels of different metabolic rate in a gradient are distinguished as high and low, or higher and lower ends or levels.

According to Boveri ('01 a, '01 b) the micromeres arise at the basal pole, and the micromere region gives rise to the mesenchyme, but Garbowski ('05) has shown that polarity of cleavage and of the later stages does not always correspond to the axis indicated by the pigment ring in *Strongylocentrotus lividus*. The elongation of the blastula occurs in the direction of the apico-basal axis, and the apical pole of the blastula represents the apical pole of the egg. Before gastrulation, the basal region of the blastula wall becomes thicker than the apical (fig. 1). In the gastrula (fig. 2) the apex of the conical body represents the apical end of the major axis, and the blastopore region the basal end, while the invaginated entoderm represents a still more

basal region of the egg and blastula, and the mesenchyme the most basal region.

The transformation of the gastrula into the pluteus begins with change from the radially symmetrical form of the early gastrula to a bilaterally symmetrical form. The basal outline of the gastrula becomes somewhat triangular instead of circular (fig. 3 *A*, basal aspect) and the apical region appears to shift toward one end of the longitudinal axis and becomes the oral lobe (fig. 3 *B*, lateral aspect). Meanwhile the apical end of the enteron unites with the body-wall at the point near the apical pole where the mouth develops, and the enteron is marked off into three regions, the oesophagus, the stomach-intestine and the rectum or anal region. Also during this period the skeletal rods appear, the body elongates and the anal arms begin to develop (fig 4, lateral aspect). Further development consists in the continued elongation of oral lobe, the appearance of the short oral arms and the elongation of the anal arms and of the body. The fully developed pluteus (fig. 5 *A* basal and fig. 5 *B*, lateral aspect) possesses a very definite antero-posterior axis, the anterior end being the broad end bearing oral lobe and arms, with the ciliated band running over the arms and oral lobe and the body margins between them, the posterior end, the opposite tapering end. Since the oral lobe represents the apical, and the anal surface the basal end of the original apico-basal axis, it is evident that this axis is no longer straight but curved, in consequence of the shifting of the apical region toward the anterior end. The course of the alimentary tract is in some degree a record of this change. In the analysis of the differential inhibitions it is essential to keep in mind this modification of the original apico-basal axial relations, as well as the antero-posterior relations of later stages.

METHODS OF EXPERIMENT

The reagents used in this series of experiments were potassium cyanide, ethyl alcohol, hydrochloric and acetic acids, sodium hydrate and ammonium hydrate in various concentrations. The eggs or embryos were placed in the solutions in

500 cc. or 1 l. corked Erlenmeyer flasks filled almost to the cork, and solutions were renewed every twelve hours where the period of action was longer than that. It has been previously determined that development would proceed normally in such corked flasks in well aerated water, renewed every twelve hours, provided the number of eggs or embryos was not too great, and particular care was taken in the experiments that the number should not be excessive. In the experiments involving recovery after temporary action of the reagent, lots of eggs or embryos were returned to sea water at certain intervals, with four or five changes to remove as far as possible all traces of the reagent.

THE EXPERIMENTAL MODIFICATIONS OF DEVELOPMENT

If the above statements concerning the relations between direct susceptibility, acclimation and recovery and metabolic rate, are correct, and if metabolic gradients are actual and effective factors in embryonic development, it is evident that three possibilities exist for the modification of development by the action of inhibiting agents. These possibilities are: first, direct differential inhibition of development by relatively high concentrations or highly toxic agents, in which case the degree of inhibition should vary directly with metabolic rate; second, indirect differential inhibition through differential acclimation to lower concentrations or less toxic agents, in which case the degree of inhibition should vary inversely as the metabolic rate; third, indirect differential inhibition through differential recovery after temporary action of the agent.

The experimental results realize expectation. In the forms produced by direct differential inhibition, the development of apical, anterior and median regions is more inhibited than that of basal, posterior and lateral regions, while, in the forms resulting from differential acclimation, the development of basal, posterior and lateral regions is more inhibited than that of apical, anterior and median. The forms resulting from differential recovery are, in general, similar to those resulting from differential acclimation.

In short, these experimental methods produce two opposed types of teratological forms with various gradations between them, and the production of the two types can be controlled to a very considerable degree, the differences in susceptibility in different individual eggs and different lots being the chief limiting factors in control. For convenience the forms produced are described under the four heads: direct differential inhibition, differential acclimation, differential recovery; differential inhibition with general recovery; and consideration of the question of control follows the description. The figures of the teratological forms are semidiagrammatic and are intended to show the form, axial relations, and chief structural features, details of mesenchymal distribution and slight skeletal variations being usually omitted. Wherever the distinction has seemed necessary or desirable, the middle region of the enteron has been drawn with a double contour, as its walls are thicker than those of the oesophageal and rectal regions which are indicated by single contours. All figures were drawn directly from living material.

THE FORMS RESULTING FROM DIRECT DIFFERENTIAL INHIBITION

The results of direct differential inhibition are most clearly seen where the development takes place in KCN, for little or no acclimation to KCN occurs within the short period of development from egg to larva. In NH_4OH , development also occurs without appreciable acclimation. Some degree of acclimation to NaOH occurs during development, but in alcohol and acids acclimation occurs so much more rapidly and completely than in the other agents used, that concentrations high enough to produce a large percentage of partial or total death must be employed to produce the forms characteristic of differential inhibition.

Considering in order the various degrees of departure from the normal form, the first appreciable differential inhibition appears as a slight change in the proportion of parts in the pluteus. A comparison of figures 6 *A* and 6 *B*, drawn from a very slightly inhibited pluteus, with figures 5 *A* and 5 *B*, (a

normal form) shows that in the former the oral lobe is shorter and less developed, the anal arms slightly shorter and the angles of divergence between the lateral skeletal rods and between oral lobe and arms are slightly smaller than in the latter, while the length of the body from the base of the anal arm is essentially the same. In these plutei the development of the oral lobe, which represents the apical region, is most inhibited and the anterior end is smaller in both dimensions than in the normal animal, while the posterior region is fully developed. Forms such as this, in which the angles of divergence of the skeletal rods are less than the normal, are called, for convenience, narrow-angled forms. These differences may appear in different degree in different individuals of the same lot, in some the only difference from the normal being a slightly shorter oral lobe, while in others, where the oral lobe is inhibited to a greater degree, the angles of divergence, which are indices of the development of the anterior end in the direction of the minor axes, are also smaller than in the norm. Where the degree of differential inhibition is so slight, the earlier development is merely somewhat retarded without appreciable departure in its course from the norm, and the differential inhibition begins to appear only in the later stages.

A somewhat greater degree of inhibition produces forms which attain the condition of figure 7 *A* and *B*, but develop no further. Here the larva remains small, the development of the oral lobe is completely inhibited, the anal arms remain short, and the dimensions of the anterior end, as compared with more posterior regions, are much reduced, so that the angle of divergence between the skeletal rods is also reduced as compared with the normal form. The entoderm, however, still undergoes differentiation into three parts and the mouth forms. It is evident that in such cases the development of the apical region is most inhibited and the development of the anterior end more than that of the posterior end.

Where the degree of inhibition is still greater, the differential inhibition may appear even as early as the elongated blastula stage, as a relative decrease in apical and increase in basal di-

mensions (fig. 8). The gastrula formed from a blastula like figure 8 is flatter, i.e., apical inhibition is greater than in the normal form (fig. 9). Occasionally, in the more extreme cases, the entoderm evaginates instead of invaginating, and an exogastrula results, but in such cases, so far as my observations go, at least some of the mesenchyme cells pass into the blastocoel. Figure 10 shows the beginning of exogastrulation, and figure 11, an exogastrula. Under no conditions, however, has exogastrulation been observed in more than one or two per cent of the inhibited forms.

In the more extreme degrees of inhibition, the form of the body becomes more nearly spherical, the entodermal differentiation more completely inhibited and the skeleton more rudimentary or completely absent, although, even when they are entirely unable to form a skeleton, the mesenchyme cells are not dead, but persist in the blastocoel. In most cases where a skeleton forms, the angle of divergence between the skeletal rods is narrow. Figure 12 *A* and *B*, shows a form with rudimentary narrow-angled skeleton and figure 13, a form in which the skeletal rods are almost parallel. Figure 14 is an anenteric larva in side view with rudimentary skeleton, a form resulting from an exogastrula; figure 15, an askeletal form with differentiated entoderm, and figure 16, a still more inhibited askeletal form, in which the entoderm never develops beyond the stage of the spherical vesicle attached to the body wall. In forms like figure 16, apico-basal and other axial differences in the ectoderm never appear, and the blastopore usually closes completely, so that it is impossible to determine whether the position of the entodermal vesicle represents the basal region or not, and certain cases of differential recovery to be described below will show that the entoderm may entirely lose connection with the blastopore region. In the case of exogastrulae where the entoderm is entirely lost, the askeletal forms develop into thin walled ectodermal spheres containing scattered mesenchyme cells, and without any visible axial differentiation in any direction.

In these spherical forms all axial differentiation beyond that of the gastrula is inhibited, and it is of interest to note that in

these cases the mesenchyme cells are scattered irregularly and do not aggregate in the basal region, as they do to a greater or less extent in less inhibited or normal forms. Evidently the localized differences in the ectoderm which supposedly determine the distribution of the mesenchyme are not present to a sufficient degree to be effective.

The most extreme degrees of differential inhibition are partial forms in which a portion of the body has been killed. In these cases death proceeds from the apical region, and the part which remains alive therefore consists of more or less of the basal region (Child '16 c). When such partial apical death occurs before the gastrula stage, the gastrulae are small, with a disproportionately large entoderm, though not always as large as the normal (figs. 17 and 18), gastrulation itself apparently being inhibited to some degree. When partial apical death occurs after gastrulation, the result is not essentially different. These partial basal forms show no ectodermal differentiations, remain spherical, the blastopore closes, and the entoderm remains as a spherical vesicle (figs. 19, 20, 21). As the figures indicate, they vary in size according as they represent a larger or smaller fraction of the apico-basal axis, and in the smaller forms, such as figures 20 and 21, the entodermal vesicle completely fills the blastocoel, because the whole of the entoderm, and only the basal portion of the ectoderm, remain alive.

In all these spherical forms, whether whole (fig. 15) or partial (figs. 19 to 21), the entoderm often loses its coherent epithelial character after several days of life without further development. In figure 22 this process is indicated. The cells become scattered about the blastocoel, and such forms are distinguishable from the anenteric forms resulting from exogastrulation, only by the larger amount of cellular material in the blastocoel. I am inclined to regard this disintegration of the entoderm as connected with its lack of functional activity, and it may be that obliteration of the metabolic gradient in the entoderm is also concerned. At any rate, this entodermal disintegration has been observed only in the inhibited forms, where the entoderm is cut off from the basal region and does not differentiate.

The forms shown in figures 6, 7, 12 to 16, and 19 to 21, are the final stages of development attained in the different degrees of inhibition. This does not mean that the animals die at these stages, but simply that no further development occurs, although life and more or less movement may continue for days. It is evident that the various degrees of inhibition are likewise progressive steps in the elimination of the axial relations as effective factors in development and differentiation. If the axes are fundamentally metabolic gradients this is easy to understand, for, since susceptibility varies directly with metabolic rate, the decrease in rate is greater in regions of higher than in regions of lower rate, and the metabolic gradient is therefore more or less completely obliterated by leveling down according to the degree of inhibition. When this obliteration by leveling down proceeds to a certain point, the metabolic gradient ceases to be an effective factor in development and differentiation, and the animal becomes physiologically anaxiate, and, therefore, development and localized differentiation cease, although life may continue for a long time.

THE FORMS RESULTING FROM DIFFERENTIAL ACCLIMATION

Differential acclimation to an inhibiting agent is of course preceded by a greater or less degree of differential inhibition which, however, is always less than where the action of the agent is sufficient to prevent acclimation. The effect of differential acclimation on the course of development and on the form of the body, differs in degree, according to the degree of inhibiting action and the rapidity and degree of acclimation, but the characteristic feature of the morphological modifications resulting from differential acclimation is that they are opposite in direction to those resulting from differential inhibition. The regions most inhibited in the one case show the greatest relative acceleration and over-development in the other.

Of course where acclimation is slight in degree or occurs slowly, differential acclimation may only partially obliterate the differential effects of inhibition, so that the resulting form merely shows a less extreme degree of differential inhibition than it

would if no differential acclimation had occurred. In such cases the form is not an adequate criterion, though increase in motor activity and rate of development may indicate that some degree of acclimation is taking place. But when the axial relations of differential development are the opposite of those resulting from differential inhibition, the form is sharply distinguishable from the form produced by differential inhibition, and it is with such forms that we are at present concerned.

Where the direct inhibiting action of the agent is not very great, differential acclimation does not produce any extreme modifications of form, but appears merely as a change in the proportions and angles of divergence of parts of the pluteus. Figure 23 *A* and *B*, shows a differentially acclimated pluteus in basal and lateral aspects. A comparison of this figure with figures 5 *A*, *B*, which represent the usual form of plutei raised in pure sea water shows that, in the acclimated form, the oral lobe, i.e., the apical region, is longer and broader, particularly at its apical end, the body is shorter and less slender, and the angles of divergence between the two anal arms, between anal arms and oral lobe, and between the short arms on the oral lobe is greater than in the sea water forms. Moreover, a comparison of figure 23 with figures 6 *A*, *B*, which show results of slight direct inhibition, makes the opposite character of differential inhibitory and differential acclimatory changes strikingly evident.

Not infrequently in these wide angled forms, the angle between the anal arms is somewhat wider than between the posterior skeletal rods, as in figure 24. This indicates a greater degree of disproportion in development between the anterior region and the more posterior levels than in figure 23, i.e., breadth of the anterior region is increased out of proportion to that of other levels of the antero-posterior axis, and the angle between the arms is therefore wider than that between the more posterior portions of the skeleton.

In KCN and NH_4OH , where acclimation is relatively slight and occurs so slowly that development is usually completed before the differential effects of acclimation become marked, even the slight changes of form shown in figure 23 do not usually

occur, but in alcohol and hydrochloric and acetic acids acclimation occurs much more rapidly and to a much greater degree, so that differential acclimation is often evident in the early gastrula stage. Consequently, with these agents much more extreme modifications of form can be produced through acclimation. NaOH is intermediate between these two groups as regards the forms resulting from differential acclimation.

The earliest marked indications of differential acclimation which have been observed occur in alcohol and acids, and consist in increase in the relative size and growth of the apical region of the gastrula. In acids this is very commonly a tapering prolongation of this region (fig. 25), while in alcohol it is more often rounded (fig. 26 *B*), but both forms may appear in both agents. Irregularity of outline and roughness of the external surface of the basal region of the gastrula (figs. 26 *A*, *B*), is particularly characteristic of the action of alcohol, though often produced to a lesser degree by the acids. It is evidently due to a decrease in the normal epithelial coherence of the cells in this region, but as development and acclimation proceed, it almost or quite disappears.

Where the apical region undergoes a relative acceleration in stages as early as the gastrula, disproportion between it and other parts increases as development proceeds. Figures 27 and 28, are side views of prepluteus stages showing this disproportion. As compared with the normal form at this stage (fig. 3 *B*) the apical and anterior regions are relatively greatly over-developed, and basal and posterior regions, including skeleton and arms are greatly under-developed. These alterations are exactly the opposite of those resulting from differential inhibition (fig. 7 *B*). In the acids, where acclimation occurs more rapidly and the differential features are more marked than in any other agents used, the most extreme modifications of this sort occur. Figures 29 and 30, *A* basal, *B* lateral aspect, show cases from acid in which there is enormous over-development of apical, as compared with basal regions, and in which the anterior region is so much over-developed that the arms approach or attain an angle of 180° , while in more posterior regions the angle of divergence,

although wider than normal, is not as great as that between the arms. In these cases the effect of differential acclimation has been extreme in the apical and anterior regions, but relatively less in more basal and posterior parts. Figures 31 to 36 show the final stages of development attained in the more extreme degrees of differential acclimation to acids. Figure 31 *A* is the basal and figure 31 *B* the lateral aspect of a larva with large oral lobe, small body and no arms. The body is short and broad and very wide angled. Evidently apical and anterior regions are relatively over-developed, basal and posterior relatively under-developed. In figure 32 *A* (basal) and *B* (lateral), these modifications are more extreme. The oral lobe is relatively longer, the body shorter and broader, and the angle of divergence wider. Figure 33 shows a still more extreme degree of modification, *A* being the basal, *B* the lateral and *C* the anterior aspect, and figure 34 shows the most extreme modification possible in this direction, *A*, *B*, and *C* being, as before, basal, lateral and anterior aspects. Here the oral lobe is larger than the body, and the skeletal rods are united to form a single straight rod lying transversely, and passing anterior, instead of posterior, to the anus. The angle of divergence between the rods is here 180° . These forms in figures 31 to 34 differ from those in figures 29 and 30 in that the angles of divergence of the skeletal rods are uniform throughout, instead of being wider anteriorly than posteriorly. This means that in figures 29 and 30 the differential effect of acclimation is more extreme anteriorly than posteriorly, while in figures 31 to 34 it has extended far enough posteriorly to determine a uniform angle of divergence of the whole skeleton, instead of a wider angle anteriorly than posteriorly. In figures 35 and 36, *A*, *B*, *C*, forms similar in outline and axial relations, but without skeleton, are shown.

It is evident that all these forms are the result of relative differential accelerations along the axes. Acclimation has made the slope of the axial gradients steeper; i.e., the regions of highest metabolic rate in each gradient have now a relatively much higher rate, and the regions of lowest rate a relatively much lower rate than under normal conditions. Consequently the greatest de-

gree of over-development, as compared with the norm, is in the regions of highest rate, i.e., apical and anterior, and apparently also median; and the greatest degree of under-development is basal, posterior and apparently lateral. In consequence of these changes in relative metabolic rate along the axes, the larva is transformed from the antero-posteriorly elongated normal form (figure 3 A) into a broad, transversely flattened, form with short antero-posterior axis; and all stages of this transformation appear.

In cases where the degree of inhibition is somewhat greater, differential acclimation in acid gives rise to forms like figure 37, which shows basal (A), lateral (B), and anterior (C), aspects of a characteristic acid form. In this larva the ciliated band, which, in the normal larva and the less modified forms, extends as a continuous band around the margins of the anterior end and over the oral lobe and basal arms, is differentiated only in the apical and basal regions, as indicated in figure 37 by the shaded bands. The apical portion of the band extends around the rudimentary oral lobe and a short distance basally, while the basal portion extends from the medial anterior region posteriorly more than half way around the body, and the lateral portions, which normally connect these apical and basal portions, are not present. In fact, the basal portion of the ciliated band in such cases forms a more or less complete ring around the basal region. This condition recalls the condition in the more primitive type of echinoderm larvae where several ciliated bands surround the body. Apparently in such cases as this, where the antero-posterior axis and bilaterality are practically obliterated, the local metabolic conditions determine that the ciliated band shall develop around the basal region, instead of over the arms and around the lateral margins of the anterior region. This condition also appears frequently in recovery forms (pp. 85, 87, 88).

When the direct inhibiting action of the agent is somewhat more lasting, and acclimation occurs somewhat more slowly, as in alcohol, the changes resulting from differential acclimation are, so to speak, superimposed on the changes resulting from differential inhibition. In such cases, therefore, various combina-

tions of differential inhibition and differential acclimation appear. The form shown in figure 38, for example, is, in its basal aspect (*A*); a characteristic case of differential inhibition, resembling figure 12 *A*, with the inhibition decreasing from the anterior to the posterior end, so that the body is relatively narrower and the angle of divergence of the skeletal rods less than in the normal (fig. 3 *A*).

The side view of this larva, fig. 38 *B*, is very different from that of the differential inhibition, figure 12 *B*. The oral lobe is large, with a rounded knob on its apical end, and the ciliated band develops only in this apical region. Great elongation in the apico-basal axis has occurred, and the enteron is attached at the mouth region, but not at the anal, and the rectal portion is absent.

Figure 39 is a side view of a form in which differential acclimation follows a somewhat greater degree of inhibition and consists chiefly in the apical outgrowth and the broadening of the anterior end.

The observation of many similar cases has shown that the course of development in cases of this sort is as follows: during the earlier stages of development the action of the agent is directly inhibitory, with the usual differential effect, but, at about the gastrula stage, differential acclimation begins to be apparent as a relative acceleration of development in the apical region, as in figures 25 and 26 *B*. From this apical region the relative acceleration progresses basally, but is more marked in anterior than in posterior, and apparently also more in median than in lateral regions. The basal region undergoes acclimation so much less rapidly and less completely than the apical that it shows no secondary changes before development comes to a standstill in the solution.

When the apical elongation begins the entoderm remains attached to the region where the mouth is to form, and it may break its connection with the blastopore region, either through closure of the blastopore (which often occurs in inhibition) or by actual rupture and so is carried apically and remains closed posteriorly, and the rectal region is absent. In short these larval

forms show differential acclimation in the more apical regions and differential inhibition in the more basal.

Figures 40 to 46 show the final stages of differential acclimation in alcohol after still greater degrees of inhibition. In all figures the apical end is uppermost, but the antero-posterior axis has been obliterated to such an extent that in most cases it is impossible to distinguish anterior and posterior regions. The most apical portion of the ciliated band is differentiated in figures 40 to 43, but in figures 44 to 46 differentiation ceases at an earlier stage. The different positions, degrees of differentiation, and relations of the entoderm depend upon the relations between the time when apical acclimation began, upon the degree of differentiation of the entoderm and upon the degree of direct inhibition. Where the direct inhibition does not bring about closure of the blastopore (fig. 15), the entoderm may separate between stomach-intestine and rectal regions, the latter remaining at the basal end and undergoing degeneration (fig. 40), or elongating and maintaining its normal basal connection (fig. 41). In other cases the blastopore closes completely, and the entoderm loses connection with the blastopore region, but remains in contact with the apical region of the body wall, and is carried with it when elongation or enlargement occur. Under these conditions it may differentiate completely, or the rectal region (fig. 40), or mouth and oesophagus (figs. 41, 42) may be absent, or lastly, it may remain a rounded vesicle (figs. 43 to 46).

Where the entoderm remains a closed vesicle it often undergoes degeneration after a few days (figs. 47, 48) as in differential inhibition (p. 74), leaving anenteric forms which may remain alive for several days longer.

Even though no skeleton develops in these forms, more or less aggregation of mesenchyme in the basal region occurs as in normal development, while, in the more extreme degrees of inhibition without acclimation, the mesenchyme remains irregularly scattered in the blastocoel. Evidently in acclimation the apico-basal metabolic differences are sufficient to constitute effective factors in determining the localization of the mesenchyme, while a sufficient degree of inhibition makes them ineffective.

If this localization is chemotactically determined, as is commonly believed, then we may say that a certain difference in metabolic rate along the apico-basal axis is necessary as a basis for the differential chemotactic action.

Attention must be called to the possibility that, in some of the forms shown in figures 40 to 48, the original apical region has been killed before acclimation, and that the apical outgrowth is a reconstitution from more basal regions. It is perfectly certain from the observations on earlier stages that forms like figures 40 to 43 may develop without any apical losses, but it is probable that at least in some cases forms like figures 44, 46 have lost some apical cells. In forms such as figures 38, 39, 40 to 48, which are very characteristic of acclimation in alcohol, the acclimation involves chiefly the more apical regions of the body, while in the basal regions the effect of inhibition persists. The difference between these forms and the acid forms (fig. 41 to 37) is due to the fact that in acids differential acclimation begins earlier in the regions of highest metabolic rate of the various axes and is more complete than in alcohol, therefore the extremes of the resulting form changes are greater than in alcohol. In alcohol differential acclimation overcompensates the effects of differential inhibition only in the more apical regions of the apico-basal axis, and differential acclimation along the antero-posterior axis plays little or no part in determining the form, while in acids the differential effect is merely greater in the apico-basal axis and is also marked in the antero-posterior and medio-lateral directions.

The lower limits of differential acclimation are reached in the partial basal forms where a considerable portion of the apical region of the ectoderm has been killed by the direct inhibiting action of the agent. Without acclimation, such forms remain spherical, the blastopore usually closes and the entoderm becomes a closed vesicle in the blastocoel (figs. 19 to 21), but, under conditions which permit some degree of acclimation, they give rise to forms like figures 49 to 53. In these a small apical outgrowth may occur with the mouth at its base, presumably on its anterior side (fig. 49); the mouth may lie at the apex of

an apical elongated region (fig. 50); an apical outgrowth may occur and mouth and oesophagus fail to differentiate (fig. 51); or mouth and oesophagus may develop in the absence of an apical outgrowth (figs. 52, 53). Usually in these cases the enteron is not connected with the blastopore region, and the rectal portion does not differentiate (figs. 49 to 51, 53), but occasionally in acids complete differentiation of the entoderm has been observed (fig. 52). Except for the case of figure 52, which has been seen only in acclimation to acids, these forms occur in both alcohol and acids.

In these cases of limited acclimation following a rather high degree of differential inhibition (figs. 40 to 53), the antero-posterior axis and bilaterality have been almost or quite obliterated as effective factors in development and, except for the position of the mouth in some cases (figs. 40 and 49), do not appear in differential acclimation. The larvae remain almost completely radially symmetrical and move with the apical end in advance as long as they live. Only the apico-basal axis remains effective, and the metabolic gradient, which constitutes this axis, has been levelled down to such an extent that it is much less effective in determining and localizing differentiation than under the usual conditions. These partial acclimations, as well as the more extreme differential inhibitions, show very clearly that the minor axial gradients can be leveled down and obliterated to such an extent that they do not reappear, while the apico-basal gradient may still remain effective to some extent. This can only mean that these minor metabolic gradients are less permanently recorded in the protoplasmic substratum (Child, '15 c, pp. 33-35), and this in turn must mean that the high ends of these minor gradients represent lower metabolic levels than the high end of the apico-basal gradient, i.e., the apical region. This conclusion is in agreement with all the facts. If the apical region is the region of highest metabolic rate in the individual, it follows of course that the high ends of gradients in other directions must have a lower rate than the high end of the apico-basal gradient; moreover, the death gradient is much more distinct in the apico-basal axis than in other directions (Child,

'16 c), and the minor axes are less effective as factors in localization and differentiation, or become effective later than the major axis. This differential obliteration of axial gradients is one of the most significant results of these experiments, and the forms in which only the apico-basal gradient remains as an effective factor in development approach in certain respects the more primitive types of echinoderm larvae.

THE FORMS RESULTING FROM DIFFERENTIAL RECOVERY

In recovery after temporary action of an inhibiting agent, a distinction must be made between the general and the differential effect. Where the effect of the inhibiting agent has not been so great that recovery is impossible, all parts of the body undergo an increase in metabolic rate after return to water. This is the general effect. The differential effect appears in the differences in rate and degree of recovery along the axes and is like the differential effect in acclimation; i.e., the higher the metabolic rate, the more rapid and complete the recovery, but since in the developmental stages of the sea urchin recovery, like acclimation, follows and is superimposed on the differential effects of inhibition, the forms resulting from differential recovery, like those resulting from differential acclimation, may show various combinations of differential inhibition and differential recovery. Since recovery involves not merely the differential changes in metabolic rate, but a great increase in rate in all parts after removal of the inhibiting agent, development may proceed farther, if the temporary inhibiting action be not too great, than where the agent is present throughout. This difference is responsible for certain interesting features of recovery which will be considered below.

Unfortunately the data concerning recovery are not complete. The close of the breeding season of *Arbacia* put an end to the experiments when only KCN and alcohol had been used in this way, but, since the results with these agents are similar to the results of differential acclimation, there is every reason to believe that the same would be true for alkalies and acids.

Where inhibition is slight and limited to the earlier stages of development, differential recovery may appear as elongation of the apical region in the gastrula (figs. 54, 55) or in prepluteus stages (fig. 56). Such forms give rise to plutei like the acclimation plutei of figures 23 and 24 with very large and long oral lobes, wide angles of divergence, and short body.

From forms of this sort, the gradation, through various combinations of differential inhibition and differential recovery, to forms which show no differential recovery is complete. Certain features of these forms require some consideration.

Where the inhibition is so great that minor gradients are almost or quite obliterated, while the apico-basal gradient still remains an effective factor in development, recovery produces a series of forms resembling the differential acclimation forms of figures 37 and 40 to 53. Figures 57 to 73 show examples of differential recovery after a considerable degree of inhibition by cyanide. In all cases the minor axes are almost or quite obliterated, but there is usually elongation in the apico-basal axis, and in many cases (figs. 57 to 64) an apical outgrowth, a rudimentary oral lobe, on which the apical portion of the ciliated band differentiates. Frequently also the basal portion of the ciliated band differentiates as a more or less complete basal ring (figs. 57 to 62, 65 to 72) as in figure 37. Here, as in differential acclimation (p. 79), the differentiation of the basal portion of the ciliated band as a partial or complete basal ring is associated with the almost complete obliteration of the antero-posterior axis and of bilaterality by the differential inhibition. This basal ring appears much more frequently in recovery than in acclimation, because, after a given degree of inhibition, differentiation proceeds somewhat further when the animals are returned to water than in the continued presence of the inhibiting agent. The development of the apical outgrowth and the apico-basal elongation are features of differential recovery, but the basal ciliated ring is a consequence of general recovery (or acclimation, fig. 37) following a degree of differential inhibition which almost or quite obliterates the minor axes. Both apical outgrowth and basal ciliated ring may be present (figs. 57 to 62)

or the basal ring (figs. 63 to 64), the apical outgrowth (figs. 65 to 72), or both (fig. 73) may be absent. It is of interest to note that those forms with well developed basal ciliated ring move with basal end in advance almost as frequently as in the opposite direction.

In some of these forms the entoderm shows normal relations and undergoes normal differentiation (figs. 57, 65, 66), but more usually the blastopore closes completely, and the entoderm loses connection with the basal region, but remains attached to the apical region. Under these conditions a mouth may develop and differentiation of the entoderm into the three parts may occur (figs. 63, 67 to 69); and in such cases the rectal region either ends blindly (figs. 63, 68, 69), or occasionally a new anal opening arises, apparently wherever the rectal region is in contact with the body wall (fig. 67). In other cases mouth, oesophagus, and stomach-intestine are present, but the rectal region fails to develop or, perhaps, becomes separated from other parts and degenerates (fig. 61), as in some cases of acclimation (see p. 81). In still other cases mouth and oesophagus may be absent; but stomach-intestine and rectal region may differentiate (figs. 58, 59, 73), or the entoderm may show little or no trace of regional differentiation (figs. 60, 64, 70, 72). Occasionally the entoderm remains in contact with the blastopore region, though completely closed and without regional differentiation (fig. 64), and a few individuals with evaginated entoderm (figs. 62, 71) resulting from exogastrulation (p. 73) occur.

These various degrees of development and relations of the entoderm depend on the degree of inhibition, the time when apico-basal elongation begins and perhaps on other factors as well. In the more extreme degrees of inhibition the blastopore usually closes completely and the entoderm becomes a closed vesicle. If acclimation or recovery occurs in such cases the entoderm usually becomes attached to the apical region of the body wall, and its further development depends on conditions in this region of the body wall and on the degree of entodermal inhibition. In some cases, where the blastopore does not completely close, the entoderm may reach the apical region and retain

its normal relations, or rupture may occur between stomach-intestine and rectal region, the latter being left in connection with the blastopore and usually degenerating. The exact history of each particular case can be determined only by isolation and continuous observation of individuals, and this I have not attempted.

Although no skeleton develops in forms of the character of figures 57 to 73, there is in nearly every case, as in similar acclimation forms, a distinct aggregation of mesenchyme cells in the basal region where the skeleton normally arises, and the conditions which determine it are evidently the same as in acclimation (p. 81).

It is probable that at least many of the forms in figures 57 to 73 have lost some part of the apical ectoderm by apical partial death, resulting from the direct differential action of the agent, and that the apical outgrowth is therefore, in the strict sense, a reconstitution of the apical region. It is certain that such forms may develop, both where a part of the apical region has died and where there have been no apical losses by death, so that the question, whether the apical outgrowth is a reconstitution of the apical end from cells which were originally not apical, has little significance. Physiologically, it is a reconstitution, whether apical losses have occurred or not, for with a sufficient degree of inhibition the apical cells themselves are incapable of developing an oral lobe, but when their metabolic rate rises to a certain level, in consequence of differential acclimation or recovery, they become capable of such development, and, since they acclimate or recover more rapidly than cells below them, the development may take the form of a local reconstitucional outgrowth.

These cases of recovery are of the same general type as the cases of differential acclimation in figures 37, and 40 to 46. In both groups the antero-posterior axes and bilaterality are almost or quite obliterated, and the apico-basal axis remains as the chief or only effective factor in orderly development.

As noted above (p. 79) forms of this kind recall, in various ways, the more primitive types of echinoderm larvae. In the

crinoid larva, for example, the blastopore closes completely, the entoderm forms a closed vesicle in the blastocoel and the anus breaks through at another point. All of these conditions, even the new anus (fig. 67), appear in these recovery forms of *Arbacia*. Likewise the basal ciliated ring recalls the ciliated rings of the crinoid larva. The facts suggest that in the primitive echinoderm larva, the apico-basal axial gradient is not as well developed as in *Arbacia*, and the antero-posterior and bilateral gradients are very slight, or become effective only in later stages. By means of differential inhibition, which decreases the slope of the apico-basal gradient and almost or quite obliterates the minor gradients, we produce axial relations which resemble, in certain respects, those of the primitive forms in which the axial gradients are less developed; i.e., less permanently recorded in the protoplasm and therefore less effective as factors in orderly development.

Figures 74 to 81 show the lower limits of differential recovery. In all, except perhaps figures 74 and 75, there has been some apical loss. In figures 74 (*A*, anterior, *B*, lateral aspect) and in figures 75 to 77, all recoveries after alcohol, the apical outgrowth develops the ciliated band characteristic of the oral lobe. In recovery after KCN, the ciliated band usually does not differentiate where the degree of differential recovery is so slight. Figures 78 to 81 show cases in which no oral lobe is differentiated, but a mouth develops apically on an elongated apical region (figs. 78, 79), or the body remains spherical and the development of the mouth is the only evidence of differential recovery. The development of the entoderm shows the same variations as in figures 57 to 73. These forms are essentially similar to the cases of differential acclimation in figures 49 to 53.

The various degrees of differential recovery in figures 57 to 81 are the final stages of development attained. Such larvae may live for a week or two and may show marked motor activity, but they do not develop further. The only reason which can be assigned for this failure to resume development, is incomplete recovery, and this means that the metabolic gradients have been obliterated or levelled down to a greater or less degree

by the differential action of the inhibiting agent, and that recovery does not completely restore the usual metabolic relations along the axes; consequently the metabolic differences are less effective in determining localization and differentiation than in the normal animal, or they are effective only in the apical region, where recovery is most rapid. It is evident that energy for development is available, for, in general, the greater the degree of inhibition of development, the longer the life after recovery before the animals die of starvation. It is not then that energy, but rather that the pattern or plan of energy distribution which the axial metabolic gradients afford is altered or more or less completely obliterated.

FORMS RESULTING FROM DIFFERENTIAL INHIBITION WITH GENERAL RECOVERY

Where the degree of inhibition is so great, the effect so persistent, or the period of inhibition so late in development that differential recovery does not completely compensate or over-compensate the morphological effects of inhibition, the general recovery which follows return to seawater often serves to bring out the differential effect of inhibition on the form and proportions of the larva more clearly than does continued action of the inhibiting agent. This is simply because development always advances somewhat farther after the animals are returned to water, and so the differential inhibitions may be carried to later stages, than in the presence of the inhibiting agent.

High concentrations of KCN, acting for a sufficiently long period at or before the gastrula stage, give particularly interesting results in this respect, because the inhibiting effect of high concentrations of KCN is relatively persistent and is therefore less completely compensated by differential recovery, but there is no doubt that similar results can be obtained with NH_4OH and NaOH , and even with acids, if the degree of inhibition be sufficient and the period of inhibition late enough in development to prevent the occurrence of differential recovery before the axial relations are fixed.

Narrow angled plutei like figures 6 *A* and *B*, are very characteristic results of this procedure, but more extreme alterations of the relations of parts are also of frequent occurrence. Figures 82 to 87 (*A* basal, *B* lateral aspect), show characteristic cases. In all of these the apical region is more inhibited than the basal, but the more complete development of skeleton and arms brings out more clearly the modification of form than where the inhibiting agent is present throughout development. In figure 13, a case of differential inhibition with practically parallel skeletal rods was shown. Figure 82 *A*, *B* shows the type of pluteus produced where such a degree of inhibition is the result of temporary action of a high concentration followed by return to water. Here the longitudinal rods and arms are parallel; i.e., the metabolic differences between anterior and posterior regions have been obliterated to such an extent that both are of the same width. In figure 83 *A*, *B*, a somewhat different modification occurs in that the two anal arms are fused to form a flat, tapering structure and the arm rods approach each other anteriorly, while the posterior portions of the skeleton still show some divergence. Figure 84 is another case of fused arms, but with parallel posterior skeletal rods, figure 85 *A*, *B*, shows a still more complete fusion of the arms and figures 86 *A*, *B*, and 87 *A*, *B*, cases in which the skeleton is median in position. In figure 86 the rectal region and anus are absent, and in figure 87 the entoderm evidently has lost connection with the blastopore region, but is fully differentiated, and the rectal region is in contact with the posterior body wall, though an anal opening could not be found.

These forms show the changes in position and relations of parts resulting from the differential effects of inhibition on the minor axes. With decreasing metabolic difference between anterior and posterior ends, the skeletal rods become more or less nearly parallel, and, in the transverse direction, the inhibition is evidently greatest in the median region, for the width of the body decreases, and structures, normally lateral, approach the median line and finally become median as the degree of inhibition of normally median parts increases.

Such larvae as these show the extremes of modification in the direction opposite to the differential acclimations in figures 29 to 36, and the extremes in the two groups represent the limits of possibility. In the one case, figures 82 to 87, parts, normally lateral, approach the median line, while in the other, figures 29 to 36, parts normally longitudinal become transverse. And yet these extreme differences in form are brought about by altering the metabolic differentials along the axial gradients, in the one case by decreasing the differences in metabolic rate along the axes through differential inhibition, in the other by increasing these differences through differential acclimation.

One other interesting feature appears very frequently in cases of recovery, particularly after KCN, where the inhibition has not been extreme, but the differential effect is not fully compensated by differential recovery. This is an extreme development of skeletal structure as indicated in figures 88 to 90. The skeleton of the anal arms in such cases is composed of many partially fused rods, the basal part of the body-skeleton and the posterior spiny enlargements may be of enormous size (figs. 88, 89), and additional short rods may arise from the regions of junction of the different rods, and even at the median junction of the two transverse basal rods, as in figure 89. Figure 90 shows a multiplication of arm rods in a more inhibited form. No attempt has been made to figure the more extreme cases of this skeletal over-development. In some the body seems to be largely filled with skeletal structures, and very aberrant shapes often result from the outgrowth of rods in various directions. In such cases skeleton development seems to have run wild. This skeletal over-development is undoubtedly a result of differential inhibition, but of course occurs only in cases where general recovery is brought about by return to water after temporary inhibition.

It has already been pointed out that the susceptibility of the mesenchyme cells is relatively low, as their origin from the basal region of the embryo might lead us to expect. This being the case, they are less affected by slight temporary inhibition than are the more susceptible parts, and therefore, when the metabolic rates of all parts are raised on return to water, the

metabolic rate of the mesenchyme cells is relatively higher, as compared with other parts, than in uninhibited animals. Because of this difference, they are able to obtain more nutrition from other parts and so to undergo more growth and division and, finally, to give rise to a larger amount of skeletal substance than normally. The normal metabolic relations between the mesenchyme and other parts have been altered to the advantage of the mesenchyme cells by the differential inhibition. Since the mesenchyme rises from the basal region of the egg, this effect of differential inhibition upon the mesenchyme is merely a special case of differential inhibition along the apico basal gradient, the mesenchyme cells (or the region from which they arise), being less inhibited because of lower metabolic rate. Since the mesenchyme cells do not remain a part of the apico basal axis, but are distributed in certain relations to other parts, the effect of differential inhibition in this case is simply an over-development of the skeleton to which they give rise.

THE CONTROL OF THE DIFFERENTIAL MODIFICATIONS

Eliminating, as far as possible, changes of temperature, constitution of sea water and other variable conditions, the control of the results with any one of the agents used is a matter of concentration, stage of development at which the inhibiting action begins, and, in the case of temporary action of the agent, the length of the time of action. Where the agent begins to act at a sufficiently early stage of development, higher concentrations produce differential and general inhibition; lower concentrations, differential acclimation, and the shorter the period of action the higher the concentration required to produce a particular modification. In experiments on differential inhibition and differential acclimation, all six agents,—KCN, C_2H_5OH , HCl, CH_3COOH , NH_4OH , and NaOH—were used, but in the study of differential recovery after temporary action, only KCN and alcohol were employed, for the close of the breeding season of *Arbacia* terminated the work before the data could be completed.

As regards general character and direction of modification of form, no specific differences appear in the action of the different agents used, but the limits of differential modification along any particular axis differ somewhat with different agents, because the relations between differential inhibition, acclimation and recovery are somewhat different for different agents, presumably because they act on the living protoplasmic system to different degrees or in different ways. With KCN, for example, all degrees of differential inhibition are readily produced by either continuous or temporary action, but KCN is so highly toxic to living protoplasm, and its effects are so persistent that, even in low concentrations, the degree of acclimation during the period of development is much less than with alcohol and acids, and, even in recovery after temporary action, if the concentration of KCN is high enough to produce any marked degree of differential inhibition, this is not usually entirely compensated by differential recovery. In other words, the differences in metabolic rate along the axial gradients are more completely and more permanently levelled down and obliterated by KCN than by alcohol and acids. KCN then is the most satisfactory agent among those used for producing axial differential inhibitions of development, free from, or but little complicated by differential acclimation or by differential recovery.

Next to KCN in this respect stands NH_4OH . While it is much less powerful as a protoplasmic poison than KCN, it is much more effective in producing differential inhibition than differential acclimation, because acclimation occurs so slowly that development is either completely arrested by differential inhibition, or, in low concentrations, is completed before any great degree of differential acclimation occurs.

NaOH acts distinctly as an inhibiting agent on these marine forms, though higher concentrations than of NH_4OH are necessary to produce a given differential effect. Acclimation occurs more readily than to NH_4OH and consequently greater differential effects of acclimation on the form and proportions are possible with this agent than with either KCN or NH_4OH .

Alcohol and acids act as inhibiting agents in sufficiently high concentration, but are less effective in producing differential inhibitions of development than any other agents used, because in almost any concentrations which do not actually kill, acclimation begins within a very short time, and, by the time development has reached the limit determined by the conditions of experiment, the primary differential inhibition is compensated or in most cases over-compensated to a high degree by differential acclimation. Acclimation to acids occurs even more rapidly and to a greater extent than to alcohol. Consequently, with alcohol and acids, the more extreme types of differential inhibition are best obtained by the action of high concentrations, beginning at the blastula or gastrula stage instead of at the beginning of development, for this procedure leaves little time for the occurrence of acclimation. Alcohol and acids, acting from the beginning of development, are the most effective of all agents used in producing the more extreme types of differential acclimation, and the acids are somewhat more effective than alcohol. The differential effects of recovery after the temporary action of alcohol are, like those of KCN, similar in character to the differential effects of acclimation and there is every reason to believe that the same is true of acids.

It is evident from these facts that the different reagents used may be arranged in a series according to their differential effects on development and larval forms. The agents which are most effective in producing the differentially inhibited types of form are least effective in producing the types of form characteristic of differential acclimation, and *vice versa*, and between the extremes of KCN and acids, NH_4OH , NaOH and alcohol may be placed in the order given. So far as the observations go, the relations as regards differential recovery after temporary action are the same as for differential acclimation. These differences in action of different agents depend upon the rapidity and degree of reversibility of their effects on protoplasm with either continuous or temporary action. I am inclined to believe that these differences in effect upon development form and proportions may be regarded as constituting to some extent a cri-

terion of the toxicity of the different agents. The more toxic the inhibiting agent, the less rapidly and completely are its differential inhibiting effects reversed, either in its presence or after its action.

As regards the relation between the stage of development at which the inhibiting agent begins to act and the concentration or period of action required for a particular effect, it is evident that, since susceptibility increases very greatly during early development up to the gastrula stage (Child, '15 b, pp. 412-418), earlier stages require higher concentrations or longer periods than later stages for the production of a given differential effect. Moreover, since the later the stage at which action begins the less time there is for differential acclimation or recovery, it is evident that the more extreme degrees of differential acclimation and recovery are most readily produced by action on the earlier stages. Where the action of the agent begins at the beginning of development and continues throughout, the result, of course, depends merely on the concentration and toxicity of the agent, and the same is true for action beginning at any particular stage, though, as already noted, the later the stage of development up to the gastrula, the lower the concentration necessary to produce a particular effect.

As regards concentration, it should be noted that in the case of KCN, when the period of action is only a few hours, the physiological effect does not increase proportionally to the concentration above a certain limit. For periods of four or five hours or less, for example, the physiological effect of KCM $m/100$ is not very much greater than that of $m/1000$, but with lower concentrations and with longer periods of action, the effect is much more nearly proportional to the concentration. No attempt has been made to determine the factors concerned in these relations between concentration and physiological effect of KCN solutions, but there are several possible factors, viz., increase in alkalinity, decrease in dissociation, with increase in concentration, the time necessary for penetration and perhaps others. Whether similar relations exist for very high concentrations of other agents used, has not been determined. These are ques-

tions of interest, but not directly connected with the chief purpose of the investigation.

One variable factor in the results, viz., the individual differences in susceptibility in different eggs of the same female and of different females cannot be controlled in these experiments. In consequence of these differences the differential effect of a particular concentration and period of action of a particular agent is different in degree in different individuals and in different lots of eggs. Since large numbers of individuals are used in each experiment a certain range of variation in the resulting forms appears, but this is not great enough to obscure the general effects. Some of the characteristic data for the different agents used follow.

Potassium cyanide

In the most extensive series of experiments KCN was used as the inhibiting agent, both continuously during development and for short periods. The forms produced show differential inhibition of greater or less degree as the characteristic feature, and the degree of differential acclimation or recovery is slight. The differential effect of KCN on the axial gradients is very persistent, and, even though general recovery may occur on return to water, there is but little differential recovery during the period of larval development. The following data will serve to indicate the range of concentrations and periods of action.

Eggs placed in KCN $m/50000$ at first cleavage and kept in this concentration continuously, develop slowly to the blastula stage where in some lots of eggs 25 to 30 per cent die completely, apical end first, while in 25 to 40 per cent death begins apically, but more or less of the basal region remains alive. In other lots practically all remain alive, at least basally, but in a larger or smaller percentage more or less apical death occurs. The final stages attained show all degrees of differential inhibition, but only rarely the slightest indications of differential acclimation.

Development from the first cleavage in KCN $m/100000$ gives little or no partial or total death and the forms produced show in most cases only slight differential inhibition like figure 6. In

HCN $m/200000$ the early stages of the plutei may show a slightly inhibited oral lobe, but later stages are usually normal in form. Development in concentrations higher than $m/50000$ usually ceases at the blastula stage and death occurs, the apical region dying first, and concentrations below $m/200000$ have no appreciable effect on development except, perhaps, a slight retardation.

In recovery after the temporary action of the higher concentrations of cyanide there are, of course, many cases in which the more apical regions are killed and only a larger or smaller part of the basal region recovers. Such cases may undergo more or less reconstitution, according to the stage of development, and may produce plutei. The following data give some idea of concentrations and periods of action.

Unfertilized eggs, 3 hours in KCN $m/1000$, then washed and fertilized. Practically all develop normally and produce normal plutei. Any differential inhibition which may have occurred in earlier stages is compensated by differential recovery.

Unfertilized eggs $6\frac{1}{2}$ hours in KCN $m/1000$, then washed and fertilized, 60 to 70 per cent develop, many with abnormal cleavages. In nearly all a larger or smaller portion of the apical region dies and is lost before the gastrula stage is reached; i.e., such portions have been so far inhibited that they are incapable of recovery.

The partial forms undergo more or less reconstitution and the resulting forms show all degrees of differential inhibition with general and some degree of differential recovery superimposed (figs. 6, 57 to 73, 82 to 90) and, as the extreme type, spherical basal forms without reconstitution (figs. 19 to 21).

Unfertilized eggs $8\frac{1}{2}$ hours in KCN $m/1000$, then washed and fertilized: 40 to 50 per cent show some development, but 20 to 30 per cent of these die before the gastrula stage, and more or less loss of the apical regions occurs in others. The forms produced show the same range of variety as in the preceding lot, but the percentage of the more extreme types of differential inhibition with less differential recovery is greater.

In these cases of inhibiting action of KCN before fertilization, the period of recovery is the whole period of development,

and during this period the lesser degrees of differential inhibition may be more or less completely compensated by differential recovery, so that the final form may approach the norm, although, in the earlier stages, there is marked differential inhibition or even apical loss. Where the degree of inhibition and therefore the apical losses are greater, differential recovery brings about more or less apical reconstitution, but in the most extreme cases which remain alive and develop, there is no reconstitution, but only a partial basal form is produced. In consequence of these various possibilities, the range of variation in form in a single lot of eggs is greater than where the inhibiting agent acts on later stages.

Eggs placed in KCN $m/20000$ – $m/10000$ at the beginning of cleavage usually continue to develop slowly, reaching the early blastula stage in 12 to 18 hours, but by the time they reach this stage their susceptibility has increased (Child, '15 b, pp. 412–418) to such an extent that further development is inhibited. If returned to water at this time a considerable percentage of total and of partial apical death occurs, and the final stages show all degrees of differential inhibition, with differential recovery in the form of apico-basal elongation and apical reconstitution (figs. 57 to 81) in a varying percentage. The same forms may be produced by a few hours in KCN $m/10000$ at the blastula stage. In fact, for any concentration which is low enough to permit development to proceed to the blastula stage, the effect is essentially the same, whether the eggs are placed in KCN at early or late cleavage and left there until they reach the blastula stage, or are placed in the same concentration at the blastula stage.

Late cleavage or early blastula stages in KCN $m/10000$ never develop beyond the blastula stage. On return to water after 36 hours in this concentration, development proceeds with a varying percentage of partial apical death, but few total deaths. Such series give the usual degrees of differential inhibition, with more or less differential recovery in the apical region in 25 to 50 per cent, appearing as apical reconstitution in some of the partial forms. With a very short period of action, very high

concentrations of KCN may be used on blastula or gastrula stages, e.g., $m/100$, 2 to $2\frac{1}{2}$ hours, $m/200$, 4 to $4\frac{1}{2}$ hours. With such concentrations and times total death occurs in 75 to 90 per cent, and a greater or less degree of apical partial death in the remainder. The partial basal forms which remain alive undergo more or less reconstitution, according as the living basal portion represents more or less of the apico-basal axis. The resulting forms show mostly the more extreme degrees of differential inhibition. A small percentage of narrow angle plutei (fig. 6) or forms with parallel or fused arms occur (figs. 82 to 87), also some forms with apical reconstitution (figs. 57 to 81), but 50 per cent or more of those which remain alive never develop beyond the spherical stage (figs. 19 to 21).

It is evident from these data that a wide range of concentrations of KCN may be used without altering the results very greatly, except as regards the percentages of deaths and of different forms. Within certain limits the chief difference is that, the higher the concentration, the larger the percentage of total deaths and partial deaths and so the smaller the percentage of living forms and the larger the percentage of partial forms which show no differential recovery or only apical reconstitution. Where the whole development occurs in KCN, differential acclimation is very slight, and occurs in very few individuals.

Ammonium hydrate

In the case of NH_4OH much higher concentrations than of KCN must be used to produce differential axial effects on development, but in concentrations high enough to produce such effects, differential inhibitions with no appreciable differential acclimation result. Eggs developing in NH_4OH $m/10000$ from the first cleavage produce practically normal forms, the only indication of differential inhibition being a slightly reduced oral lobe.

Eggs developing in NH_4OH $m/5000$ from first cleavage, show 25 to 50 per cent of total deaths and most of the remainder show partial apical death. 80 to 90 per cent are spherical basal forms like figures 19 to 21, without reconstitution or differential

acclimation. The remainder show various degrees of differential inhibition, the least inhibited being small plutei like figures 12 and 13. The various differential inhibitions are of the same types throughout as those produced by KCN, and no forms of any other sort appear in such cases.

Sodium hydrate

NaOH is much less toxic than NH_4OH . Concentrations above $m/500$, acting continuously from the first cleavage, usually inhibit development or kill completely at the blastula stage. In $m/500$ from the first cleavage, a considerable percentage of total deaths occurs at or before the blastula, and most individuals show more or less apical death. The resulting forms show differential inhibition, usually of the more extreme types, and many remain spherical basal forms like figures 19 to 21.

In $m/1000$ from the first cleavage, more or less apical partial death may occur in 10 to 40 per cent, but few or no total deaths. Of the resulting forms the majority show some degree of differential inhibition, but some are normal plutei, and some show a slight degree of differential acclimation. All degrees of differential inhibition occur, and differential acclimation appears only in the relatively large oral lobes and wide angles of divergence in some plutei (figs. 23, 24, 29).

In $m/2000$ from the first cleavage, apical partial death may be almost entirely absent, or it may occur in 15 to 20 per cent of the blastulae and ranges from the death of a few apical cells to death of about the apical half. A few of the partial basal forms, in which apical death has proceeded farthest remain spherical (figs. 19 to 21) or show some apical reconstitution, but the great majority of the embryos form plutei, almost all of which show the relatively large oral lobe characteristic of differential acclimation, and in 5 to 10 per cent the angles of divergence are wider than normal (figs. 23, 24, 29).

In $m/5000$ from the first cleavage, the effect on both rate of development and on form is slight. Apical death does not occur, and all form plutei which are either normal or possess a slightly larger oral lobe and slightly wider angles than normal.

NaOH in sufficiently high concentration evidently retards or inhibits development and produces all degrees of differential inhibition in form. Differential acclimation occurs more rapidly and to a greater extent than in NH_4OH , but still is not sufficient to produce the more extreme types of form characteristic of differential acclimation in alcohol and acids.

Alcohol

Development from the first cleavage in alcohol 4 per cent is greatly retarded, and apical death begins at or before the blastula stage, usually resulting in total death before or in the gastrula stage, but in a small percentage more or less of the basal region may remain alive in some lots and a few small spherical, partial forms like figures 20 and 21 may result.

In alcohol 3 per cent from the first cleavage, total death occurs in 20 to 50 per cent in the blastula or gastrula stage, and most of the others show more or less apical death. The resulting forms show the more extreme types of differential inhibition, and mostly remain without a skeleton.

Development is retarded in 2 per cent alcohol, but there is usually no death, and differential acclimation begins with apical outgrowth as early as the gastrula stage (figs. 25, 26), and the final stages reached are forms like figures 40 to 48, without skeleton and with an elongated apical outgrowth representing the oral lobe, with a few like figures 49 to 53, which represent partial basal forms.

In 1.5 per cent alcohol development is less retarded and the degree of differential acclimation is greater. About half the resulting forms show marked elongation and over-development of the oral lobe like figures 27, 28, 38, 39, and the remainder range through the forms of figures 40 to 48.

In 1 per cent alcohol plutei develop which range in form from slight degrees of differential inhibition to slight degrees of differential acclimation, and in lower concentrations there is usually no effect on form.

A few experiments on recovery after the temporary action of alcohol indicate the possibilities in this direction. Late cleavage stages in alcohol 4 per cent 11 hours, then washed and re-

turned to water, advanced to the blastula stage during the alcohol period, but no movement occurred until returned to water. At the end of the alcohol period partial death, ranging from a few apical cells to the apical half had occurred in from 25 to 40 per cent. The resulting forms are all without skeleton and show differential inhibition of the more extreme degrees, with apical differential recovery indicated by apical outgrowth, as in figures 74 to 81, in 30 to 40 per cent. The remainder, mostly partial basal forms, are spherical with entodermal vesicle (figs. 19 to 21) and show no differential recovery.

Late cleavage stages in 3 per cent alcohol, 18 hours, advance to beginning of gastrulation during this period, but 10 to 20 per cent show a small amount of apical death. Resulting forms are from 30 to 40 per cent differentially inhibited plutei with more or less rudimentary skeleton like figures 7, 12, 13, and with no evidence of differential recovery; 30 to 40 per cent are askeletal rounded forms with apical outgrowth like figures 74 to 81; and the remainder are spherical with entodermal vesicle, mostly partial basal forms, without differential recovery. This case shows an interesting feature of the relation between differential inhibition and differential recovery. Those individuals which are least inhibited develop most rapidly and so attain an early pluteus stage before differential recovery occurs to any great extent. Consequently they show only the lesser degrees of differential inhibition. Those which are more susceptible are more retarded in their development, and even after return to water attain a less advanced stage of development, but, nevertheless, show a greater degree of differential recovery than the less susceptible, because renewed growth and development are limited to the apical region, while in the less susceptible individuals they occur more or less over the whole body. Finally, in the most susceptible individuals, more or less of the apical region is killed, and differential recovery does not occur to any appreciable extent. In short, the forms characteristic of differential recovery can be produced only within certain limits of inhibition. If the degree of inhibition be not sufficient or be too extreme, the degree of differential recovery is not sufficient to affect the form of the animal.

Acids

Development from the first cleavage in HCl $m/1000$ proceeds slowly, reaching the blastula stage in about 24 hours; with 50 per cent more or less of deaths, mostly total. During the next two or three days the mesenchyme cells enter the blastocoel, and after four or five days, gastrulation may begin. Even in this concentration the gastrulae begin to show apical outgrowths like figures 25, after six days in the solution, but do not usually develop further, and even if returned to water at this time they die in a day or two.

In HCl $m/2000$ from the first cleavage, a few total deaths occur and more or less of the apical region dies in 20 to 40 per cent at or before the blastula stage. From this stage on, differential acclimation begins, and the whole gastrulae are like figure 25, the preplutei, like figures 27 and 28, and the final stages range from the extreme types of differential acclimation shown in figures 31 to 36, through skeletal forms with apical outgrowth representing the oral lobe and with or without basal ciliated band, like figures 37, 60, 61, 64, and rounded forms with small apical outgrowth like figures 49, 51 to the lower limits of differential acclimation like figures 50, 52, 53, 78 to 81. In fact, practically every individual which lives through the gastrula stage, even as a partial basal form, shows at least some degree of differential acclimation.

In HCl $m/5000$ from the first cleavage there are usually no deaths, and apical outgrowth begins in the gastrula (fig. 25), and the resulting forms range from wide-angled plutei with large oral lobe (figs. 23, 24, 29 to 32), through normal plutei, to inhibited forms with apical acclimation (figs. 37, 60, 61, 64, 49, 51). The extreme types of differential acclimation like figures 33 to 36 do not usually appear in this concentration.

In HCl $m/10000$ from the first cleavage, all develop into wide angled plutei with large oral lobe, about 50 per cent approaching normal, like figures 23 and 24, and the remainder ranging from these to forms like figures 29 to 32.

In HCl $m/50000$ from the first cleavage, 50 per cent, more or less, are normal, the remainder slightly wide angled with large

oral lobe, with forms like figure 23 as the maximum modification of form.

Acetic acid was used only for comparison with HCl, and in two concentrations $m/2000$ and $m/5000$, acting continuously from the first cleavage. The forms produced were of the same type and range of variation as in the same concentrations of HCl.

With the acids, as with alcohol, it is evident that for the more extreme modifications of form by differential acclimation, a certain degree of differential inhibition is necessary as an antecedent condition. The most extreme modifications of form by differential acclimation appear in acids because differential acclimation begins earlier and progresses more rapidly than in other agents, so that the relations of parts are widely altered before the skeleton develops.

GENERAL CONCLUSIONS

The experimental data leave no possibility of doubt concerning the effectiveness of the axial metabolic gradients as fundamental factors in the embryonic development of the sea urchin. The differences in susceptibility to inhibiting agents which are associated with the differences in metabolic rate at the different levels of the gradients, determine on the one hand the differential effects of direct inhibition and, on the other, those of differential acclimation and differential recovery.

Since there are no specific differences in the form-changes produced by the different agents used, there is no basis for the assumption of specific action in any case. All the facts indicate that the action of the various agents is essentially quantitative, so far as it concerns the processes of growth and development. It is very probable that the different agents do not all act in exactly the same way on the sea urchin protoplasm, but the point of present importance is that, however they act, whatever condition or reaction complex in the system they affect primarily, their general effect is a retardation or inhibition of the fundamental metabolic processes, which may be more or less completely reversed by acclimation or recovery. The changes in form result from the differences in effect on different regions,

and these in turn are dependent upon the differences in rate of reaction or in protoplasmic condition, permeability, aggregation, enzyme activity or whatever designations we prefer, associated with the differences in rate of reaction. The relation between susceptibility, and metabolic rate is a very general one, and is apparently independent, at least to a large extent, of the particular component reaction or condition of the protoplasmic reaction system which is directly affected in a particular case. It is dependent, rather, upon the fact that living protoplasm is a system and that no very great changes in any essential component of this system are possible without affecting the system as a whole.

It is evident that the alterations in form resulting from differential inhibition and from differential acclimation and recovery are very closely associated with changes in the relative rate and amount of growth at different levels of an axis. The so-called normal form of the sea urchin, or of any other organisms, represents merely the usual relations of metabolism and growth between different parts.

Experimental data of many kinds and from many fields show that where nutritive supply is limited, a region of high metabolic rate will grow or maintain itself more or less completely at the expense of a region of lower rate; i.e., some of the products of breakdown in the region of low rate go to the building up of new molecules in the region of high rate because the physico-chemical conditions determine a passage of these substances toward the region where they are being most rapidly transformed. In short, the region of higher rate of reaction robs the region of lower rate. Where the nutritive supply equals the demand in all parts, the region of higher rate of reaction shows, in general, a higher rate of growth than a region of lower rate, simply because it synthesises more molecules in a given time (Child, '15 b, Chap. II). General form of body and proportions of parts are, fundamentally, the expression of relations of this character. They represent, so to speak, the metabolic balance between regions of different rate of reaction under a particular complex of conditions. The 'normal' form is merely one par-

ticular case, or, more correctly speaking, a certain limited range of variation in metabolic relations, and it is normal, merely because, under the usual conditions, this range of variation is not exceeded.

Not only differential growth, but local differentiation, may occur at different points of a metabolic gradient. Some of the evidence in support of the view, that local differentiation results, in the final analysis, from the differences in metabolic condition which arise at different points of a gradient, in consequence of the differences in rate of reaction, has been considered elsewhere (Child, '15 c, pp. 127-169, 183-188). If this view be correct, 'normal' localization of differences in development is like general form and proportion merely, one particular case or a certain limited range of cases representing a certain limited range, the 'normal' range of variation in the essential metabolic conditions.

The experimental methods used in the present paper serve merely to alter the differences in metabolic rate between different parts and so to alter the resulting balance and therefore the spatial order as expressed in growth, form, and differentiation. In differential inhibition the slope of the metabolic gradient is decreased, the gradient is more or less completely leveled down, because the regions of high rate of reaction are more susceptible to the action of the inhibiting agent and so undergo a greater decrease in rate, than the regions of lower rate. In consequence of this decrease in slope of the metabolic gradient, the differences in metabolic rate between different points of the gradient, and therefore the differences in rate and amount of growth, become less than in the normal animal, and the relative size and proportions of parts along any axis are altered, those parts which represent regions of high rate of reaction becoming relatively smaller and those which represent regions of low rate, relatively larger. As this decrease in slope of the metabolic gradient progresses the gradient becomes less effective as a factor in differentiation, and local morphological features along its course may become less and less marked and finally disappear, or more correctly, fail to appear.

In the various degrees of differential inhibition described, the progressive obliteration of metabolic gradients, i.e., of axes appears. Bilaterality may be almost completely obliterated, while longitudinal and apico-basal gradients still remain effective (figs. 86, 87); and in other cases both bilaterality and the longitudinal axis are practically obliterated and the apico-basal axis remains as the chief determining factor in growth and differentiation. This condition is most evident where differential acclimation or recovery occurs in the apical region, but there is little or no indication of other axes in the larva. Figures 40 to 53 and 57 to 81, show various stages in this axial obliteration. The limit in this direction is the obliteration of all axes, including the apico-basal axis. This limit is approached or perhaps attained in some cases (figs. 19 to 21). Under these conditions definite progressive development and localized differentiation cease, although life may continue until ended by starvation.

In differential acclimation and differential recovery the changes in metabolic relations between different points of a metabolic gradient are in the opposite direction from those in differential inhibition. Since, with the method employed, differential acclimation and recovery are possible only after differential inhibition, it may happen that metabolic gradients are so far obliterated by differential inhibition that differential acclimation or recovery is impossible, but where the gradient is not obliterated to this extent, differential acclimation and recovery consist in an intensification, a steepening of its slope, beginning at the high end. The metabolic differences between different points of the gradients are increased, and the form and proportions of the larva and the positions of localized differentiations show changes in the opposite direction from those characteristic of differential inhibition. The apical region develops at the expense of the basal, the anterior at the expense of the posterior and the median at the expense of the lateral.

The differences between the forms resulting from differential inhibition and differential acclimation show to what extent the general form, proportions and localization of parts can be altered and controlled in this way. It is possible to transform the

larvae in one direction into apico-basally elongated forms, with slight apical development, with little or no trace of longitudinal axis or bilateral symmetry, or, in the extreme case, into a spherical form, with little or no trace of any axis, and in the other into a transversely flattened form with very great apical, anterior and median over-development, and the localization of parts differs correspondingly in the two types. The direction of the skeletal rods may be shifted through ninety degrees, or the skeleton may be completely inhibited or very greatly over-developed. It would be difficult, I think, to find a more complete demonstration of the effectiveness of the metabolic gradients in development than these form-changes in the sea urchin. We are able to modify, to control, and to predict the changes in form and relations of parts which occur.

The fact that differential effects on development result from the action of inhibiting agents, not only upon the various stages of development themselves, but upon the unfertilized egg is also important. It may appear, at first glance, that this fact constitutes a demonstration of the actual existence of the definitive axial metabolic gradients in the unfertilized egg, but this is not necessarily the case, as a moment's consideration will show. Assuming that no metabolic gradients are present in the unfertilized egg, the effect of the inhibiting agent must be the same on all parts of the protoplasm or of its limiting surfaces. If this effect persist after fertilization, the protoplasm is less capable of stimulation and therefore, if the metabolic gradients arise *de novo* during or after fertilization, it is conceivable that differences in metabolic rate in such gradients may be less than in normal eggs. If, on the other hand, recovery from the inhibiting effect is occurring at the time the metabolic gradients arise, the differential acceleration of metabolic rate associated with the establishment of a metabolic gradient may itself determine a differential recovery, since the rate and degree of recovery varies with metabolic rate. In short, a general inhibition preceding the establishment of the metabolic gradients, if its effect persist during the period of establishment, may determine that the metabolic differential, the metabolic slope of

such gradients, when they do arise, shall differ from that produced in the normal egg by the same factors. The demonstration of the existence of metabolic gradients in the unfertilized egg by this method is then not complete. The evidence from death gradients is also unsatisfactory on this point, because, with the low metabolic rate in the unfertilized egg, differences in rate are at least slight (Child, '16 c). According to Boveri ('01 a, '01 b) the apico-basal axis of embryo and larva coincides with the axis of the growing oocyte in *Strongylocentrotus lividus*, but Garbowski ('05) has shown that such coincidence does not occur in all cases, and, according to Wilson and Mathews ('95), the embryonic axis in *Toxopneustes* may form any angle with the axis determined by the position of the polar bodies and of the nucleus after maturation, while in *Asterias* these two axes coincide.

These various observations afford no adequate basis for general conclusions. If they are all correct we must conclude that in echinoderms the apico-basal axis of the embryo may coincide with that of the growing oocyte, or may be determined *de novo* by factors acting on later stages. If the original axis of the growing oocyte is a metabolic gradient, it is evidently not very strongly marked or permanently fixed in the protoplasm, and other factors acting differentially on the egg (Child, '15 b, Chaps. II, V.) may determine a new effective gradient, as is apparently the case in *Asterias* (Wilson and Mathews, '95; Child, '15 a), and as Garbowski's observations indicate in some cases in *Strongylocentrotus* (Garbowski, '05). If any metabolic gradient is present in the unfertilized egg of *Arbacia* it is apparently slight (Child, '16 c), and it seems probable that the differential conditions associated with fertilization, or perhaps, in some cases, the differential action of other external factors might determine a new effective gradient. In general, the observations, as far as they go, suggest that the apico-basal embryonic axis coincides with the axis of the growing oocyte, except where the differential action of other factors is sufficient to determine a new effective gradient, and this is very probably true for many other animal eggs.

As regards the longitudinal axis and bilaterality in *Arbacia*, the fact that they do not become visibly effective factors in development until later stages, and also the fact that they can be experimentally almost or quite obliterated while the apico-basal gradient still persists, suggest that they are less marked as metabolic gradients and perhaps determined later than the apico-basal axis. Conceivably they may arise in connection with fertilization through the differential effect on the egg protoplasm of the positions or paths of the pronuclei and the direction of the spindle axis. But at present no final conclusion is possible, and, as pointed out above, the differential effects on later development of the action of inhibiting agents on unfertilized eggs do not constitute a demonstration of the existence of the definitive axial gradients in the unfertilized eggs.

Attention has already been called to the fact that some of the differentially inhibited forms resemble, in certain respects, the more primitive types of echinoderm larvae as regards the complete closure of the blastopore, the apico-basally elongated, more or less cylindrical, conical or ovoid body without strongly marked antero-posterior axis or bilaterality, and the development of the basal portion of the ciliated band as a more or less complete basal ring (p. 79). It may also be noted that the wide-angled forms resulting from differential acclimation show some resemblances in form to certain ophiurid plutei. These resemblances suggest that the condition of the axial metabolic gradients in the experimentally produced forms of *Arbacia* and the normal forms which they resemble, is somewhat similar. The fact that certain degrees of obliteration of the axial gradients by differential inhibition produce an approach to more primitive larval types, is particularly interesting as suggesting that the evolution of the pluteus has consisted to some extent in an evolution of the axial gradients, or, more correctly speaking, of the protoplasmic conditions which determine the establishment, metabolic slope and physiological effectiveness of such gradients. The pluteus, in short, shows a higher degree of axiation than the crinoid larva, i.e., the axes appear more distinctly in form and differentiation of the body, and there is

reason to believe that certain of the minor metabolic gradients in the pluteus are not present in the crinoid larva; certainly they are not effective factors in larval development.

The modifications of form produced by differential inhibition and differential acclimation and recovery, demonstrate the reality of the metabolic gradients as effective dynamic factors in the development of *Arbacia*. The analysis of these modifications indicates that the apical region is the region of highest rate of reaction in the organism; or in other words, that the region of highest rate of reaction becomes the apical region. From the apical region the rate of reaction decreases in all directions, but less rapidly on that side which becomes anterior than on that which becomes posterior, and apparently less rapidly along the median region than laterally. The development of the skeleton of course modifies these simple relations and determines new localized regions of growth and so new local gradients.

The method of demonstrating the axial metabolic gradients by the death gradients in lethal concentrations of inhibiting agents (Child, '13 b, '14 a, '15 a, '15 c, Chap. III, '16 a) is much less delicate than the method of modifying development by differential inhibition and differential acclimation or recovery, but the conclusions reached by means of the cruder method, so far as they go, are confirmed, and further conclusions made possible by the more delicate method.

A few data not yet published on differential inhibition in the larvae of the starfish and of certain polychete annelids, indicate that the metabolic relations can be altered along at least the apico-basal gradient in the same way as in *Arbacia*. Moreover, in the reconstitution of isolated pieces of *Planaria*, differential inhibition and differential acclimation and recovery in the longitudinal and transverse axial gradients can be brought about in the same way as in the sea urchin larva, and the resulting modifications of form are of the same character. In differential inhibition anterior (apical) regions are most inhibited, posterior least, and organs normally bilateral, such as the eyes and the cephalic lobes, approach the median line and, in the more extreme degrees of differential inhibition, become median (Child,

'12, '16 b), while in differential acclimation and recovery the changes are opposite in direction. The fact that axial relations in these widely different organisms can be experimentally altered and controlled in similar ways through the differential effect of inhibiting agents indicates very clearly the fundamental identity of the physiological axes as metabolic gradients. In the light of these facts, the further demonstration of the existence of axial metabolic gradients in algae among plants (Child, '16 a) and in protozoa, coelenterates, flatworms, echinoderms, annelids, fishes, amphibia and birds among animals and their correspondence with developmental gradients of other kinds are sufficient to establish the general and fundamental significance of such gradients in the development and differentiation of organisms.¹

The modifications of form, localization, and differentiation by differential inhibition, acclimation and recovery in the sea urchin larvae and in the reconstitution of pieces of *Planaria* afford a basis for the interpretation of many other cases of experimental teratogeny and of various teratological forms observed in nature. The cases of cyclopia in fishes produced experimentally by Stockard and others are simply differential inhibitions like those produced in the sea urchin and *Planaria*. Median regions are more inhibited than lateral, and lateral organs approach the median line or become median. Differential inhibition in the antero-posterior axis in the early stages of development may produce various gradations from the normal form to acephaly. In segmented animals, where a region of high metabolic rate arises secondarily at the posterior end of the primary gradient and becomes a growing region from which new segments develop, a secondary gradient arises, with its region of highest rate in the posterior growing region, and differential inhibition along this gradient may produce certain characteristic teratological forms.

In short, the examination of teratological forms which are not the direct result of some factor acting locally, but are produced by agents or conditions which affect the body as a whole,

¹ Child, '12, '13 b, '14 a, '15 a, '15 c, '16 a, '16 b, '16 c, Hyman, '16. A considerable part of these data is still unpublished.

indicates very clearly that differential susceptibility along metabolic gradients plays a very important part in their production. And as I have shown for the sea urchin, the same agent or condition may not only produce different degrees of modification in a particular direction, according to concentration, period of action, etc., but may produce modifications in opposite directions, the one direction representing differential inhibition, the other differential acclimation or differential recovery. On the other hand, very different agents and conditions may produce similar modifications of form, because the general effect upon metabolism is not specific, but quantitative. The facts indicate that the fundamental factors concerned in these modifications are alterations in one way or another of the rates of reaction and so of the metabolic relations of parts.

As a matter of fact, experimental teratogeny affords the most conclusive evidence of any field of investigation for the fundamental significance of metabolic gradients in orderly development and differentiation, for the modification and control of development by modification and control of the metabolic rates and relations in these gradients enables us to determine and test their effectiveness. Experimental teratogeny has suffered from the failure to find a general foundation for experimental procedure, analysis and interpretation, but from the view point which we attain with the aid of the conceptions of metabolic gradients and differential susceptibility, a wide field lies before us ready for logical experimentation, analysis and synthesis.

SUMMARY

1. If the directions or axes to which the order apparent in development is related are fundamentally gradients in rate of metabolic reaction, it must be possible to alter the developmental order as expressed in form, proportion and localization of parts by altering the relations of rate of reaction along these axes or gradients.

2. The general relation between susceptibility to a great variety of agents and conditions which retard or inhibit metabolic reaction in one way or another and rate of metabolic reaction,

affords a means of altering the relations of metabolic rate along such gradients. The relation between susceptibility and metabolic rate is briefly as follows: in concentrations or degrees which kill without permitting acclimation, susceptibility varies directly with metabolic rate. In low concentrations, where acclimation occurs, the rate and degree of acclimation vary directly with metabolic rate. Where the inhibiting agent acts only temporarily the rate and degree of recovery vary directly with metabolic rate.

3. Acclimation to, and recovery from the action of the inhibiting agents used in these experiments consists in the attainment of a higher rate of reaction, either in the presence of the agent or after its action.

4. Potassium cyanide, ethyl alcohol, ammonium hydrate, sodium hydrate, hydrochloric and acetic acid in all concentrations above a certain minimum retard or inhibit development of the sea urchin, but with sufficiently low concentrations, a greater or less degree of acclimation may occur in the presence of the agent, or a greater or less degree of recovery after temporary action.

5. A graded difference in susceptibility to these agents exists along the axes of developmental stages of the larval sea urchin body. This difference in susceptibility appears either as differential inhibition, differential acclimation, or differential recovery along the axes, according to the concentration of the agent used and the period of action.

6. These differential effects along the axes bring about characteristic changes in form, proportions and localization of parts of the larval body, which can be experimentally controlled and predicted to a very considerable degree.

7. The larval forms resulting from differential inhibition show changes in form, proportions and differentiation in a certain direction from the normal, the degree of change corresponding to the degree of differential inhibition. The chief changes are decrease in size of oral lobe, which represents the apical region, decrease to zero in the angle of divergence between arms, approach of lateral parts toward the median line and in more extreme degrees fusion in the median line, the progressive oblit-

eration of antero-posterior and medio-lateral differences and finally of apico-basal differences.

8. The changes in differential acclimation and differential recovery are in the opposite direction. They consist in increase in size and over-development of the oral lobe, increase to 180° in the angle of divergence between the arms, and over-development of anterior and median as compared with posterior and lateral regions.

9. Where the effect of differential inhibition persists after general recovery, a great over-development of the skeleton may occur, since the mesenchyme cells are relatively less inhibited than most, if not all other parts.

10. Forms produced by the more extreme degrees of differential inhibition resemble, in certain respects the forms of the more primitive echinoderm groups, and the wide-angled plutei resulting from differential acclimation resemble larvae of ophiurids. These facts suggest that in the evolution of the pluteus larva from the primitive larval form, changes in the metabolic relations along the axial metabolic gradients and perhaps the establishment of conditions which determine new gradients, have played an important part.

11. All the experimental data indicate that the spatial orders in the larval development of the sea urchin are fundamentally gradients in rate of general metabolic reaction, with highest rates of reaction determining apical, anterior and median regions.

12. The different agents used differ in degree in their inhibiting action, but there is no evidence in the changes of form produced of any specificity of action. The order of decreasing effectiveness in inhibiting development is as follows: cyanide, ammonium hydrate, sodium hydrate, ethyl alcohol, acids. This order is also the order of increasing capacity of the sea urchin to become acclimated to, or to recover from the effects of these agents.

13. The relation between susceptibility and metabolic rate affords a method for analytic teratogenic investigation and a basis for the interpretation of many cases of experimental teratogeny already recorded and also of many teratological forms observed in nature.

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PLATES

EXPLANATION OF PLATES

All figures are drawn from living individuals; and form, proportions, and axial relations are reproduced as exactly as possible, but they are otherwise semi-diagrammatic in that details not essential to the purpose of the paper are omitted. The ciliated band on the anterior end and arms of the pluteus is drawn only in figures 5 *A* and *B* to indicate its normal relations and in figures 37 to 43, 47, 48, 57 to 72, 74 to 77, where its development is modified. In other figures of plutei (figs. 6, 7, 23, 24, 29 to 36, 82 to 89), where its development shows essentially the usual relation to that of the anterior end, it is omitted. The mesenchyme cells are omitted in all cases, and the fenestration of the arm rods and the posterior skeletal structure is merely diagrammatically indicated. In figures of blastulae and gastrulae, all parts are drawn in double contour to indicate the thickness of the body layers. In figures of stages intermediate between gastrula and pluteus single contours are used, except for the thick-walled stomach-intestine, and in figures of pluteus stages single contours are used throughout.

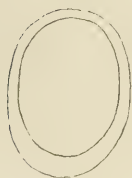
Figures lettered *A*, *B*, *C* with the same number are, respectively, basal (anal) lateral and anterior views of the same individual except in the case of figure 74 where *A* is a lateral and *B* an anterior view.

PLATE 1

EXPLANATION OF FIGURES

Normal development

1 to 5 Elongated blastula to fully developed pluteus.



1



2



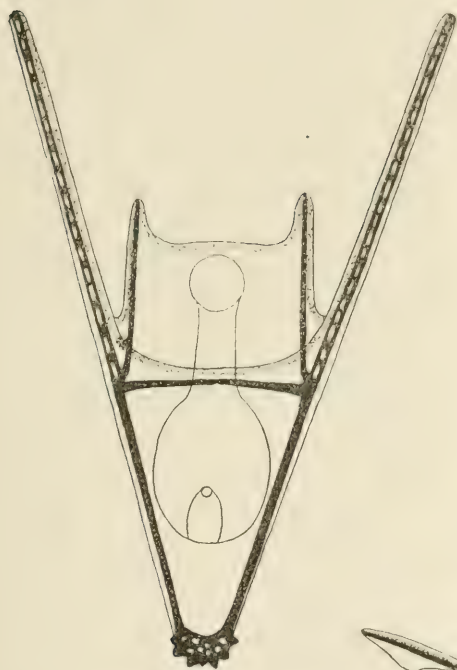
3A



3B



4



5A



5B

PLATE 2

EXPLANATION OF FIGURES

Differential inhibition. •

- 6 *A B* Narrow-angled pluteus with somewhat inhibited oral lobe.
- 7 *A B* Final stage with greater degree of differential inhibition.
- 8, 9 Differentially inhibited blastula and gastrula.
- 10, 11 Exogastrulation.
- 12, 13 Final stages of more extreme differential inhibitions showing decrease of angle of divergence of skeletal rods.
- 14 Anenteric pluteus, probably from an exogastrula.
- 15 Askeletal form.
- 16 Spherical anaxiate form.
- 17, 18 Partial basal gastrulae.
- 19 to 21 Final stages of small basal forms.
- 22 Degeneration of enteron in spherical form.

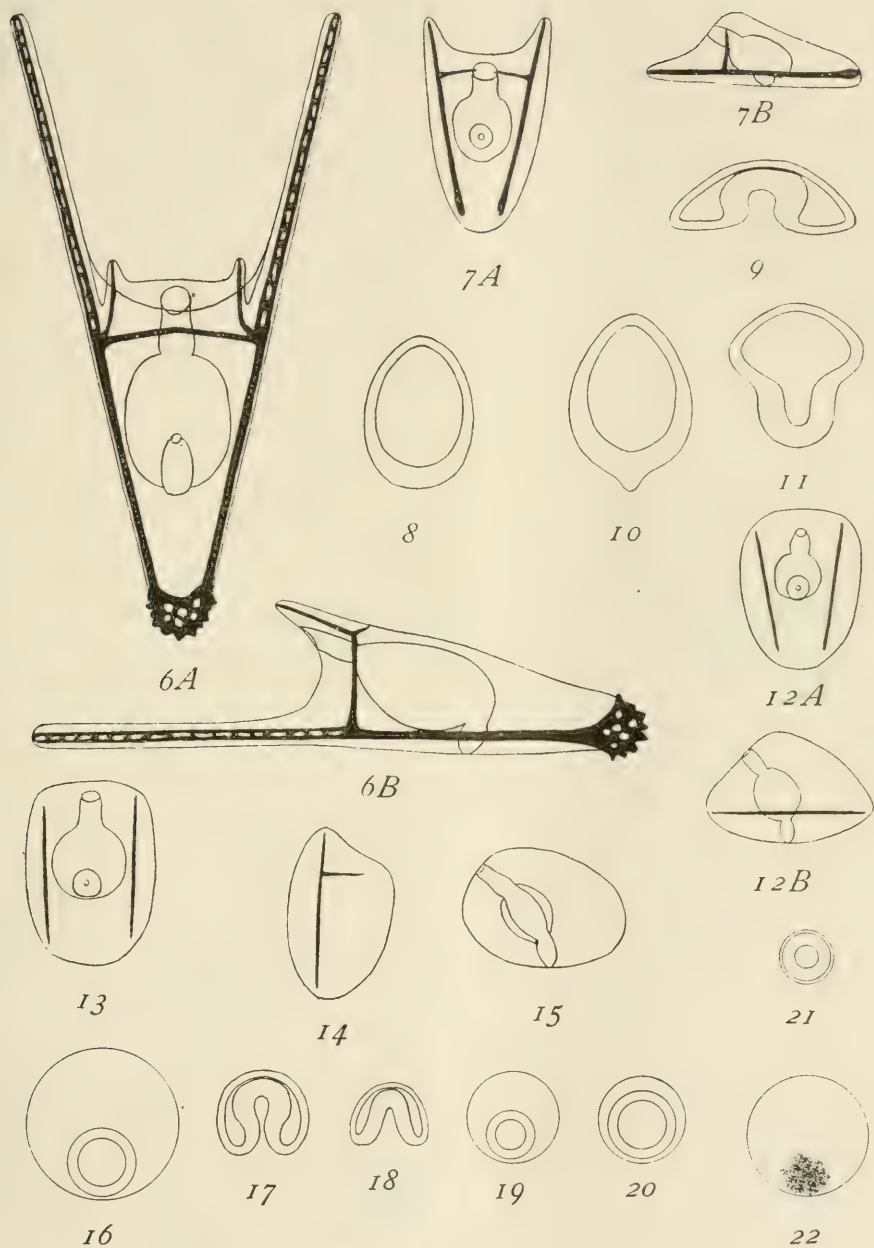


PLATE 3

EXPLANATION OF FIGURES

Differential acclimation

- 23, 24 Wide-angled plutei.
- 25 Differential apical acclimation in gastrula.
- 26 Apical acclimation with basal irregularity in alcohol gastrula
- 27, 28 Apical acclimation in preplutei.

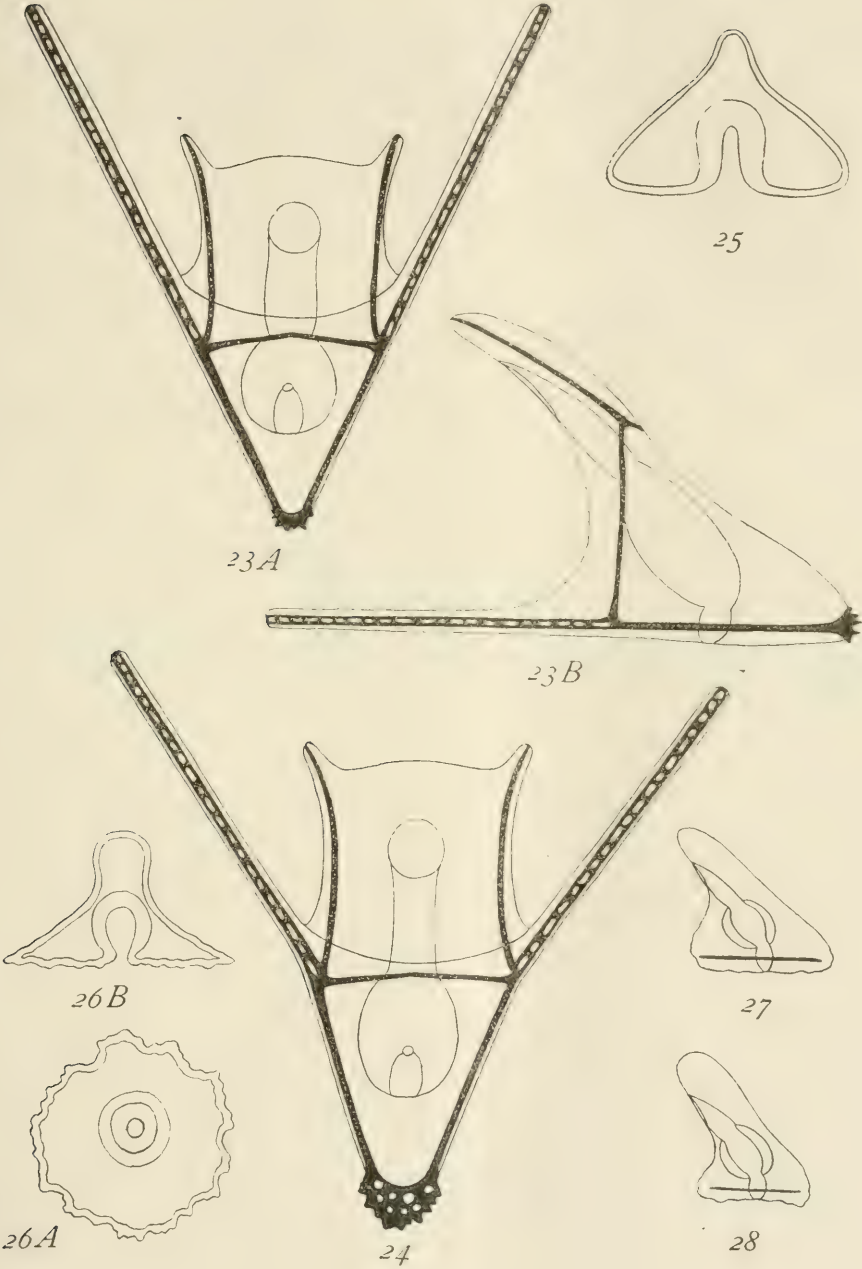


PLATE 4

EXPLANATION OF FIGURES

Differential acclimation

29 to 33 Cases of differential acclimation showing over-development of apical and median anterior regions as compared with basal, lateral and posterior regions.

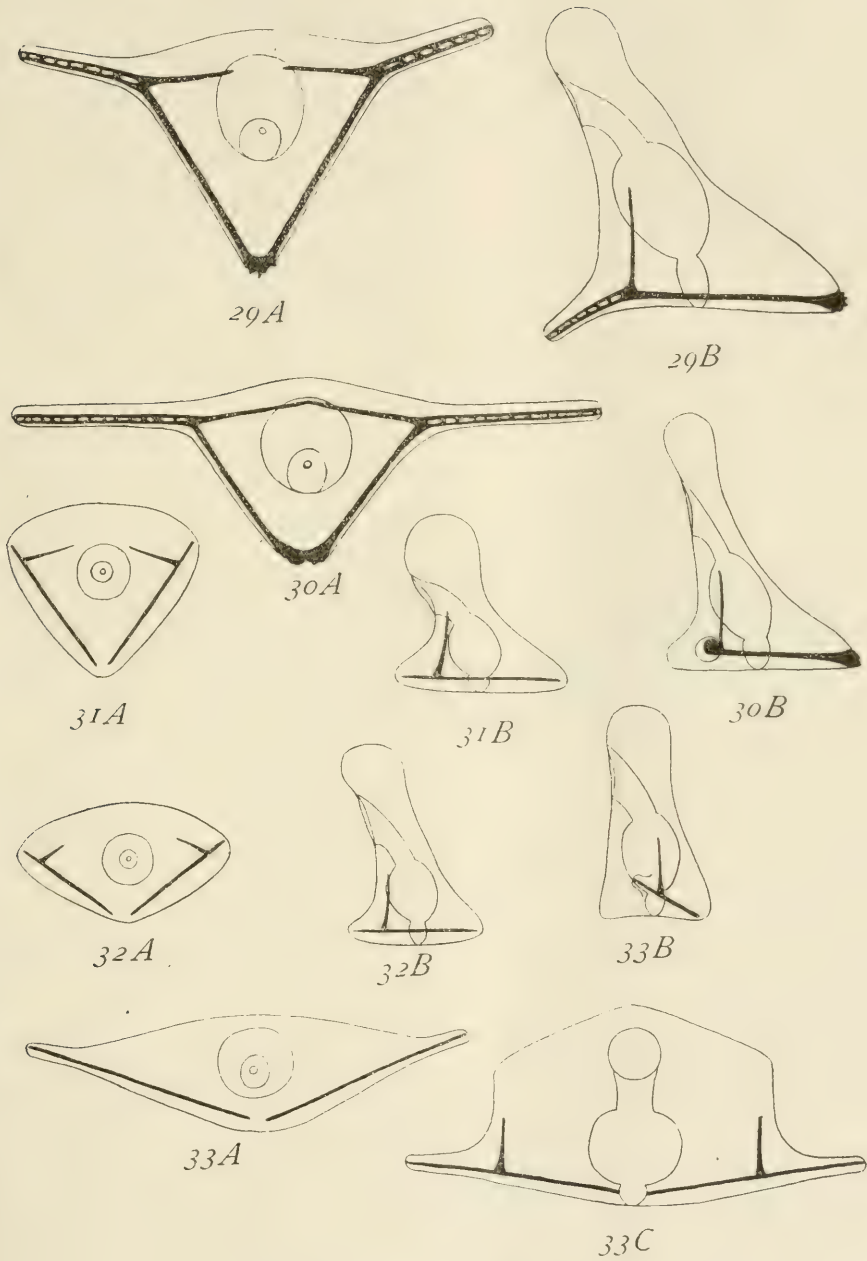


PLATE 5

EXPLANATION OF FIGURES

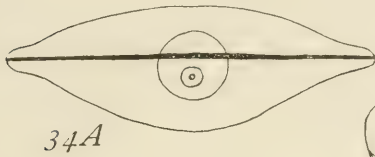
Differential acclimation

34 to 36 Further cases of over-development of apical and median anterior regions as compared with basal, lateral and posterior.

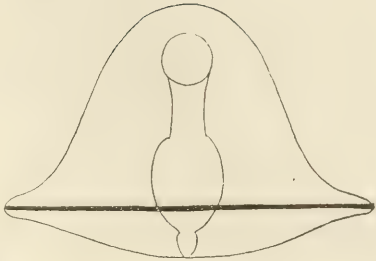
37 Differential acclimation after greater degree of inhibition, showing modification in course of basal portion of ciliated band.

38 A larva showing apical acclimation and basal inhibition.

39 Apical acclimation and basal inhibition.



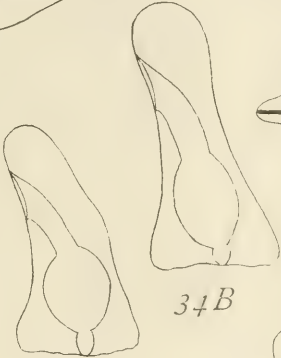
34A



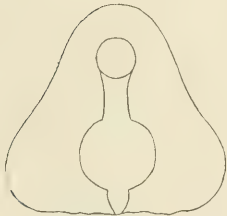
34C



35A



35B



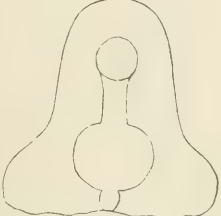
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36A



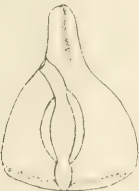
36B



36C



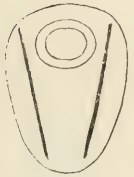
37A



37B



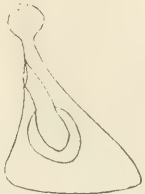
37C



38A



38B



39

PLATE 6

EXPLANATION OF FIGURES

Differential acclimation and differential recovery

40 to 48 Cases of apical acclimation after the more extreme degrees of differential inhibition, in which the antero-posterior axis and bilateral symmetry have been more or less completely obliterated.

49 to 53 Cases illustrating the lower limits of differential acclimation after extreme differential inhibition which has obliterated all axes except the apico-basal.

54 to 56 Differential recovery in early and late gastrulae and early pluteus.

57 to 62 Cases of differential recovery after a considerable degree of differential inhibition, showing rudimentary oral lobe as an apical outgrowth, and a basal portion of ciliated band; antero-posterior axis and bilaterality more or less completely obliterated: figure 62 from an exogastrula.

63, 64 Cases of differential recovery with rudimentary oral lobe, but without basal portion of ciliated band.

65 to 67 Cases of differential recovery in which oral lobe does not develop, mouth is apical and basal portion of ciliated band is present; in figure 67 the enteron has separated from the blastopore region and a lateral anus has formed.

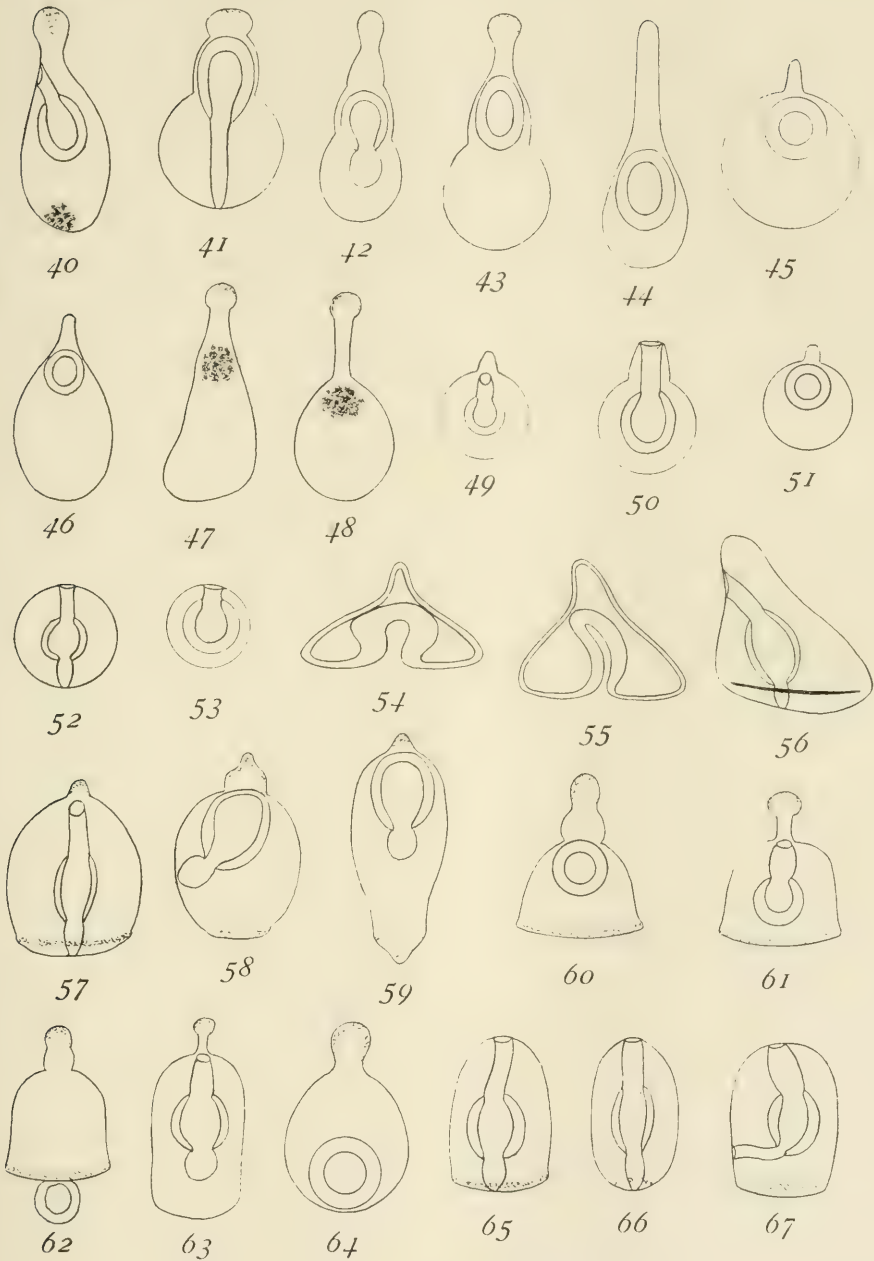


PLATE 7

EXPLANATION OF FIGURES

Differential recovery and differential inhibition with general recovery
68 to 73 Further cases of differential recovery, showing various differences in detail; figure 71 from an exogastrula.

74 to 77 Cases in which differential recovery is limited to the development of a rudimentary oral lobe; figure 77 a partial basal form.

78 to 81 The lower limits of differential recovery.

82 to 84 Persistent differential inhibitions after general recovery showing under-development of apical and median anterior regions as compared with basal lateral and posterior.

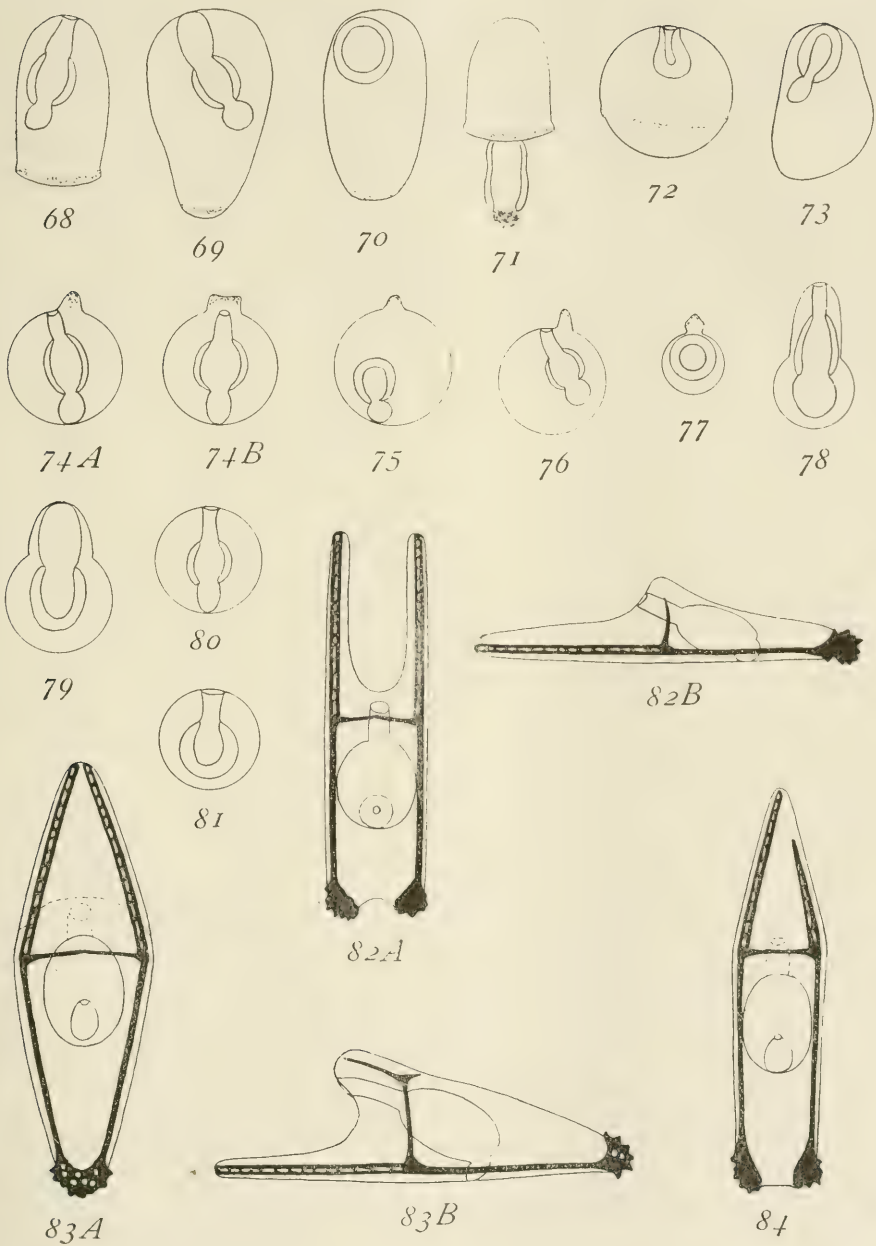


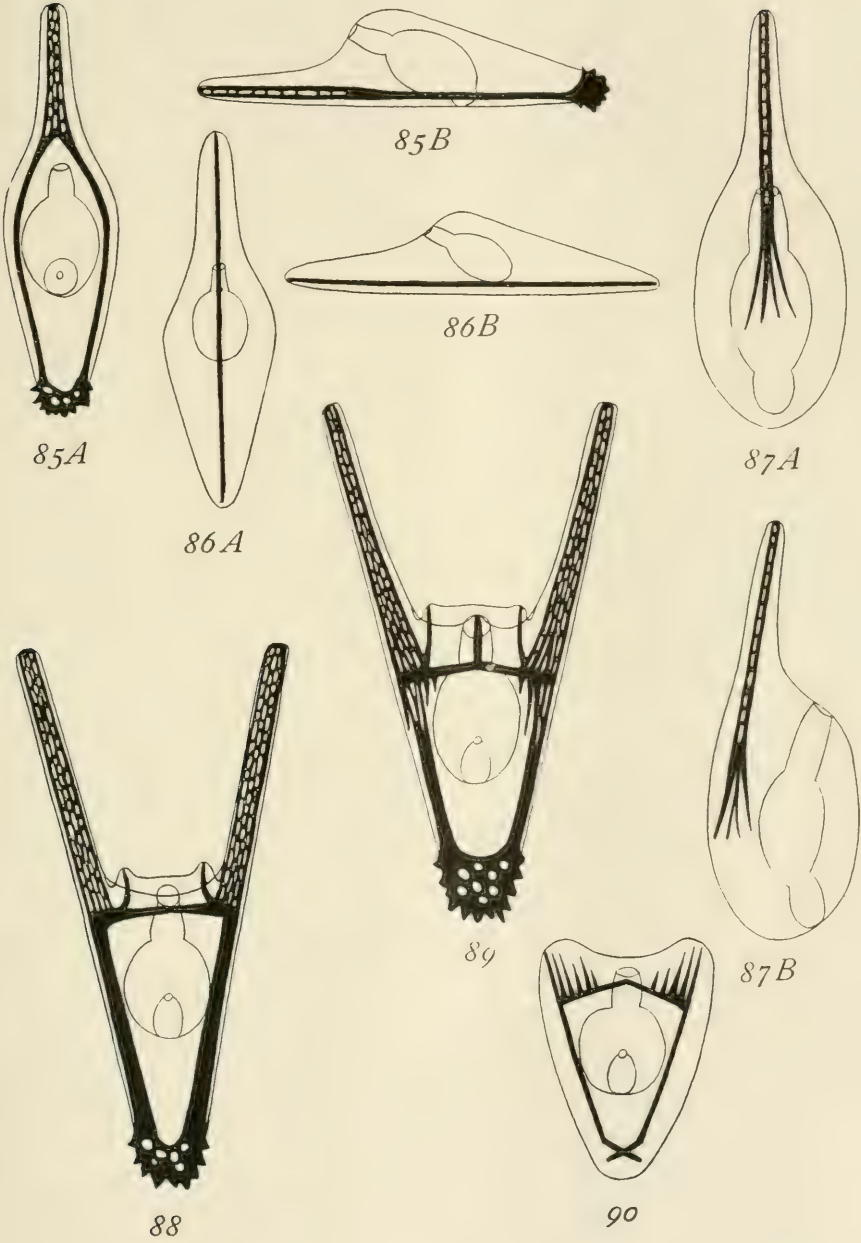
PLATE 8

EXPLANATION OF FIGURES

Differential inhibition with general recovery

85 to 87 More extreme degrees of persistent differential inhibition with general recovery.

88 to 90 Over-development of skeleton in cases of slight degrees of persistent differential inhibition with general recovery.





ON THE SINUS PARANASALES OF TWO EARLY TERTIARY MAMMALS

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FIVE FIGURES

It is generally supposed that the accessory nasal cavities of mammals have had a long history, since they develop relatively early in the embryo (Schaeffer, '10). It will be interesting to note, in one of the two very early mammals described below, the enormous development of these cavities. Fortunate casts of the sinus paranasales of an oreodont (*Merycochoerus*) and an early bear-dog (*Daphaenus*) came into my possession some time since. In view of the active discussion in regard to the morphology of these cavities in the vertebrate head, it is thought that a discussion and illustration of these, the oldest known paranasal sinuses, would be of interest. One of the casts is so perfectly formed that the small bloodvessels (rami A. ethmoidalis anterior) of the mucous membrane (*mucosa sinus paranasali*) lining the sinuses, are as sharp and distinct as if the sinuses were of a recently dissected animal, in which the arteries had been injected (fig. 1).

A review of the literature of the sinus paranasales is not necessary in this place, since this has been fully done by Kallius ('05), who lists titles of four hundred and twenty-one studies (1562–1905) dealing with this question. He has given further the best pictures of the casts of these cavities, as they occur in man, which have so far been published. The work of Schaeffer ('10), Read ('08), Dicuľafé ('06), Osborn ('12), and the writer ('15), should be noted in this connection. So far as the writer is aware, there has never been an attempt to study systematically the casts of the paranasal cavities of fossil mammals, material for which is very abundant in the various paleontological collections. It is a part of the purpose of this paper to call

attention to this phase of paleontological work, which offers considerable interest in vertebrate morphology, as well as to describe the two casts above referred to. A comparison of the



Fig. 1 A photograph of the sinus frontales and a part of the sinus maxillaris superior of the oreodont, *Merycochoerus*, to show the rich blood supply, as indicated by the molds of the arteries. $\times 2$.

results of paleontology and embryology, in regard to these cavities, would be of great importance.

The casts made by von Brunn and Kallius ('05; figs. 35 and 36) of the sinuses of the human head, beautiful and complete

as they are, are surpassed by the casts of these cavities made by nature and preserved in fossil form. They show us the complete form of the cavities, the blood supply, the thickness of the limiting walls, and the relations of the sinuses to the nasal cavity and to the brain (fig. 2).

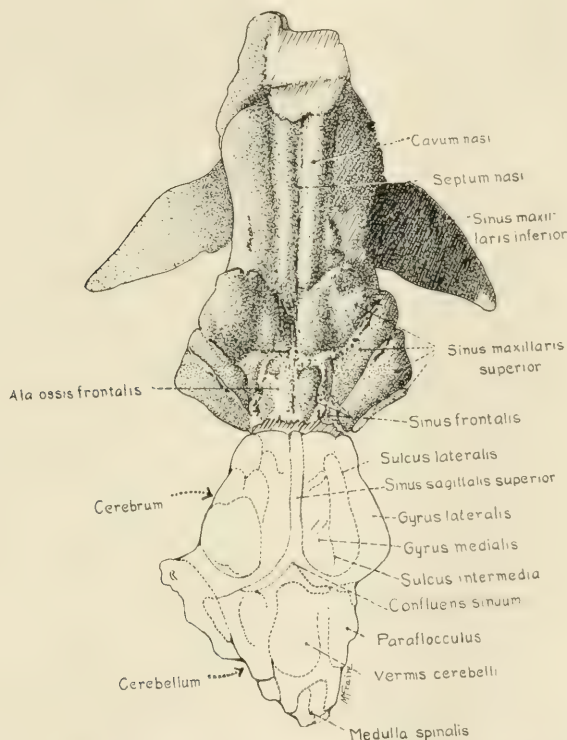


Fig. 2 Dorsal view of the casts of the brain and the sinus paranasales of the oreodont, *Merycochoerus*, from the White River Oligocene. The position of the eyes and the location of the external auditory canal renders it probable that this animal was aquatic. $\times \frac{2}{3}$.

The oreodont cast shown in figures 1, 2, and 3, represents a member of an extinct group of artiodactyls which was so abundant and so characteristically American in Oligocene and Miocene times. Scott ('13) says of these animals:

The Oreodontidae was one of the most characteristic of North American artiodactyl families, and its members were exceedingly abun-

dant throughout the Upper Eocene, the whole Oligocene and Miocene, ending their long career in the Pliocene. In distribution the family was exclusively North American and no trace of it has been found in any other continent. . . . Dr. Leidy, who first discovered and named most of the genera, spoke of them as combining the characters of camel, deer and pig, and called them 'ruminating hogs' . . .

The writer has already ('15) figured an imperfect brain cast of one species (*Merycoidodon*) of this group, and with the present nasal casts there is associated a splendid brain cast, which will be described at another time, in conjunction with other brain casts of Tertiary mammals.

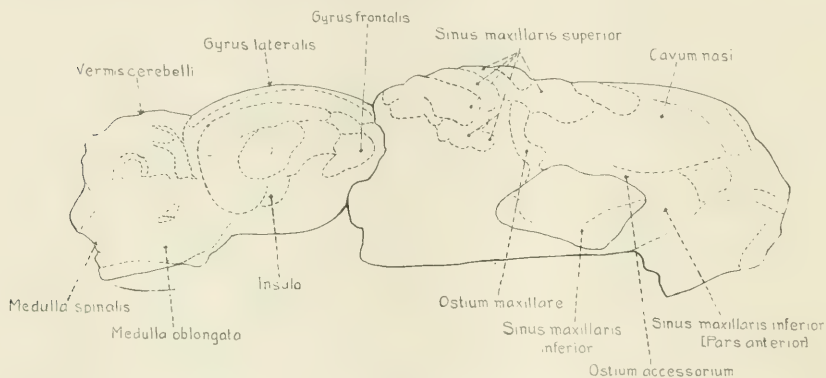


Fig. 3 Lateral view of the casts of the brain and accessory nasal sinuses of *Merycochoerus*. $\times \frac{2}{3}$.

The species represented by the present cast is a member of the genus *Merycochoerus*.¹ The position of the sinuses (fig. 4) is indicated in an outline of a skull figured by Matthew. The skull was slightly distorted by pressure and the brain and sinuses do not fit in an exact manner; but sufficiently well to show their location. The elongate nature of the maxillary sinus is evident, having a most unusual posterior extent.

Although the sagittal crest is fairly well developed in this species there are no sacculations of the frontal sinus in this animal, such as occurs in the pig (Sisson '14, fig. 180), and the ox (Sisson, fig. 135): although the diploic air-spaces were en-

¹ Matthew, W. D. 1901 Mem. Amer. Museum. Natl. Hist., vol. 1, pt. VII, p. 405.

larged to diameters of three to four millimeters. Perhaps in some of the oreodonts, such as *Promerycochoerus*, which had a much more highly developed sagittal crest, the sagittal sacculations were more highly developed. The sinuses, as they are developed in the present specimen, more nearly resemble those of the sheep than those of the pig, with which the oreodonts have

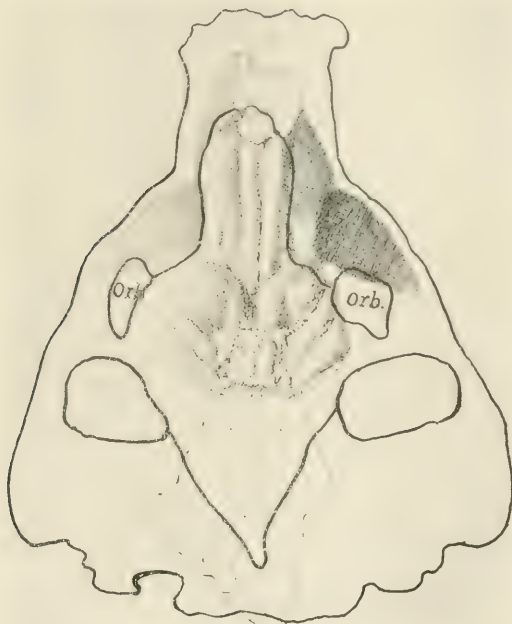


Fig. 4 Outline of the skull of *Merycochoerus* (After Matthew) superimposed on the drawings of the casts of the accessory nasal sinuses, to show the location of these cavities in the head. $\times \frac{1}{4}$.

some affinity. A skull showing the accessory sinuses of the sheep was used for comparison with the oreodont.

The group of small cavities (figs. 1, 2 and 4) just anterior to the brain, are, doubtless, all divisions of the frontal sinus. In the frontal region of a calf, Mihalkovics ('99; Taf. 5, fig. 24) has figured a sacculation of the frontal sinus, which in this fossil form has taken the shape of separate cavities. This condition of the sinuses, however, is, in the human skull, subject to such

an enormous range of variation that it cannot be said at present whether the small sinuses in *Merycochoerus* are primitive or are simply peculiar to this individual form. An extended study of casts of these animals would settle this point. None of the cavities in the fossil seem to be due to infoldings of the ethmoturbinal, but are either divisions of the frontal or maxillary sinuses. The smaller posterior, or frontal sinuses, are separated from each other by rather thick partitions of bone (*alae ossi frontali*). It is quite evident that the large cavities just anterior to the small posterior ones, are sacculations of the maxillary sinus, which have been named (fig. 2) the sinus maxillaris superior, as in the sheep and horse. The relatively great distance, thirty millimeters, between the most anterior one of the sinus maxillaris superior and the base of the sinus maxillaris inferior is traversed by a canal which is possibly homologous to the ostium maxillare. This condition is particularly parallel in the sheep, but whether it is proper to speak of this opening as the ostium maxillare, or not, is uncertain.

The divisions of the frontal sinus are small, slender, and irregularly developed (figs. 1 and 2), exhibiting characters which are, so far as I am aware, unparalleled in modern mammals. The descending wings of the frontal bone, which separate the sinus frontales from the sinus maxillaris superior, are relatively thick as in the sheep, and from the anterior surfaces of these alae in the oreodont, as in the sheep, doubtless sprang the ethmoturbinals.

There are five sacculations of the sinus maxillaris superior. They are much larger than the sinus frontalis, and their surfaces are richly supplied with blood vessels (fig. 1), which are, I suppose, branches of the A. ethmoidalis anterior. The most posterior division of this group of sinuses, is quite slender superiorly and deep posteriorly, forming a cavity which was flattened by the anterior part of the jugal arch. A lateral inferior division is pea-shaped. It is quite prominent and projects sharply into the maxillary bone.

The sinus maxillaris inferior is enormously developed, and in the cast, takes the form of two backwardly projecting horns.

These sinuses occupied the entire cavity of the maxillary bone, as in the sheep, and projected posteriorly into the zygomatic arch, to a point below the orbit. The maxillary sinuses are not so many nor so complex as in the sheep, but the posterior division of the inferior group extends much further into the zygomatic arch. There are several minor sacculations of the sinus maxillaris inferior, one below the horn-like projection. Anteriorly there is a rather large accessory sinus. Leading backward from this cavity is a canal which is possibly homologous to the ostium accessorium of human anatomy. The accessory sinus is not very prominent laterally, but has a large expanse antero-posteriorly, extending beyond the premaxillo-maxillary suture.

The two canals, which have been designated ostium maxillare and ostium accessorium, connect the two great divisions of the sinus maxillaris (inferior and superior) with the sinus frontalis, and for this reason I have supposed they have been properly designated. The surfaces of all the casts are richly supplied with blood vessels.

The brain cast (fig. 2) is very similar to that of the sheep, with which it has been compared in naming the gyri and sulci. The various divisions of the encephalon will be fully described later.

The other cast (fig. 5) is that of a bear-dog (*Daphaenus*) from the White River Oligocene of South Dakota. This cast is not so well preserved as the oreodont, and not so much detail can be discerned, but sufficient is present to warrant a description. In order that the nature of the species may be understood, it will be necessary to say that *Daphaenus felinus* is the ancestral form of a group of bear-dogs, members of the family Canidae, which appeared in well-developed form in North America during the Oligocene and Miocene times. Their ancestors are probably to be found in the Creodont family, the Miacidae, of the Uinta Eocene.

Through the Oligocene the phylum (Canidae) was carried back by the several species of *Daphaenus*, assuredly the ancestor of *Daphaenodon* and decidedly more primitive in many respects. The Oligocene genus was a much smaller animal than its lower Miocene successor,

the larger species hardly equalling a coyote; the teeth were smaller and more closely set, but the molars were proportionately large, while the carnassials were less finished and effective shearing blades. The skull was less distinctively dog-like and had a smaller brain-case, with very prominent sagittal and occipital crests, a longer cranium and shorter face; the tympanic bones were very small and so loosely attached to the skull that they are rarely found, a very striking difference from all existing dogs. The backbone was remarkable for the unusually large size of the lumbar vertebrae, a point of resemblance to the cats and suggesting that *Daphaenus* had great powers of leaping;

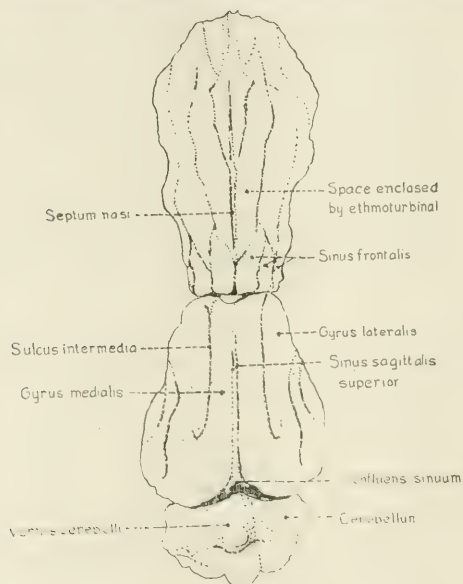


Fig. 5 A dorsal view of the cast of the brain and nasal cavity of *Daphaenus felinus*, a bear-dog from the Oligocene. $\times \frac{2}{3}$.

there was a long, heavy, leopard-like tail, and the caudal vertebrae were very like those of the long-tailed cats. The limbs and feet were similar in character and proportions to those of *Daphaenodon*; but the astragalus was less grooved for the tibia, the claws were rather more retractile and the gait was probably more plantigrade. There were so many cat-like features in the skeleton of *Daphaenus*, that the observer cannot but suspect that these resemblances indicate a community of origin, but, until the Eocene ancestors of the cats are found, the question of relationship must remain an open one (Scott, '13, p. 526-7).

The sinus frontales are present (fig. 6) in well-developed form, and are singularly like the frontal sinuses of the modern dog

(Sisson, '14, fig. 212), although the posterior superior portion of the cavities has been lost in the fossil form. Compared to the modern dog, these cavities are smaller, measuring in the present specimen fifteen millimeters in length by seven in breadth. The cavities are, however, more numerous and more elongate. There are three of them to each side of the median septum, the medial one of which is the larger and longer, while that on the lateral surface is about half the size. Thin plates of bone separated these cavities. There are no evidences of the blood supply, since the cast had been somewhat eroded before collection.

The sinus maxillaris is not completely preserved, since the portion of the cast which projected into the maxilla is lost. The base of this projection, which lies well forward, is present and it indicates a more extensively developed sinus than occurs in modern dogs. There are no superior divisions of this sinus. The impressions of the fluting of the ethmoturbinals are preserved very clearly. These impressions indicate a *cavum nasi* of larger capacity than the modern dog possesses, and likewise apparently would indicate a much keener sense of smell, although the *cavum nasi* is greatly narrowed at the point of entrance of the olfactory nerves.

The brain cast is very well preserved, especially the cerebellar portion. The form of the brain is essentially dog-like, though not so richly convoluted as in the modern dog. The cat-like characters, evident in its skeletal make-up, have made little impress on the form of the brain. Its form is quite distinct from that of the cats of the same period.

A study of these two casts leads us to believe that the origin of the sinus paranasales is to be found, not in the early mammals, but in their ancestors, and probably their remote ancestors. Just how far back we would have to go to get the phylogenetic beginning of these interesting cavities is uncertain. Their nature in the early reptiles and amphibians is unknown. Descriptive paleontology has, so far, paid no attention to these interesting phases of the morphology of extinct forms, and it is certainly well worth developing. It will not only give new life to the science itself, but will assist in the interpretation of many features of vertebrate morphology which are now obscure.

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PHOTOGENIC ORGANS AND EMBRYOLOGY OF LAMPYRIDS

F. X. WILLIAMS

TEN PLATES

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INTRODUCTION

This paper is a part of the results of two years work at the Bussey Institution of Harvard University, on the habits, structure and development of two common fireflies, *Photuris pennsylvanica* and *Photinus consanguineus*. The first of these furnished nearly all of the embryological material and the second was the more advantageous for the study of the development of the photogenic organ of the adult. Not much attention was paid to the physiological aspects of the problem.

The material obtained was studied both in the living state and in sections. Most used for killing the embryological material was hot water followed by increasing strengths of alcohol. Larvae, pupae and adults were first immersed in hot water and then in warm Kahl's fluid (Water, 30 parts; 96 per cent alcohol, 15 parts; formalin, 6 parts; glacial acetic acid, 1 part). Usually the material was imbedded in paraffin, but when the chitin was very thick, celloidin was used. Embryos were usually stained in toto with borax carmine; the sections with Heiden-

hain's iron haematoxylin with orange G, eosin or Congo red as a counter stain, the first of these proving best. Sections were cut from 3.5 to 15 micra thick. Osmic acid was much used for the study of the 'tracheal end cells,' while caustic potash brings out the tracheal capillaries very satisfactorily.

I wish to thank all who have aided me in my work and especially Dr. W. M. Wheeler and Professor C. T. Brues, under whose supervision my studies were made, for many helpful suggestions and criticisms.

ORIGIN AND DEVELOPMENT OF THE PHOTOGENIC ORGANS

There are, in general, two views as to the origin of the photogenic organs in the Lampyridæ; according to one they are developed from the ectoderm; according to the other they are related to the fat-body and are therefore mesodermal. These two beliefs rest upon a diversity of observations and facts. The development of the photogenic organs of the European *Lampyris noctiluca* has been studied by Vogel ('13), who published a rather brief paper without illustrations on the subject. There does not appear to be any fundamental difference between his observations and mine. As far as I know, Vogel's studies are the only ones of their kind.

Lindemann ('63) considers the photogenic body to be a nervous organ. This view is not remarkable when we find that he figures a part of the central nervous system as the light-organ.

Owsjannikow ('68) believes that the light-organ of *Lampyris* consists of a group of epithelial cells of a glandular nature. Presumably, like the majority of glands in insects, he considers these cells to be ectodermal.

Gegenbaur ('74), in speaking of the light-organs of fireflies, advances the view that the non-luminous or urate layer is derived from the fat-body, whereas the photogenic layer is a modification of the hypodermis.

Heinemann ('86) in speaking of the elaterid, *Pyrophorus noctiluca*, p. 298, says: "Die Leuchtorgane der Cucujo's gehören wie diejenigen den Lampyriden dem Hautsystem an."

Dubois ('98), in his studies on the light-organs of Lampyridae and of *Pyrophorus noctiluca*, found, from an examination of the ova of the Lampyrids, a resemblance between the blastoderm cells and the photogenic cells of the larva, pupa and adult. He concludes with reason that it is these cells and not the nutritive yolk which give rise to the light-organ. It would seem that some of the photogenic material is transmitted to the eggs by the mother insect. He says, p. 310:

Les noyaux se sont multipliés dans un certain nombre de gros plastides hypodermiques, et de ceux-ci s'échappent des files de jeunes éléments, dont la masse forme l'organe lumineux larvaire . . . ; plus tarde, celui-ci s'isolera: la photographie de cette nouvelle coupe, que je projette sur le tableau, vous permet de comprendre facilement sa texture.

As a result of this proliferation, the underlying hypodermis is very sparse or lacking. His figure 139 illustrates this condition. Dubois' figure of the photogenic organ of *Pyrophorus noctiluca* is rather different from that of the Lampyridae. Dahlgren and Kepner ('08), and Geipel ('15) figure a portion of the photogenic organ of *Pyrophorus*, and it can be seen that it has essentially the same structure as that of *Photuris* and *Photinus*. Dubois describes, further, the formation of the light-organ in the female *Lampyris noctiluca*, and bases his observations largely upon a pupa about to transform into an adult. The process is described as essentially similar to that which occurs in the larva, the photogenic cells being derived from the underlying hypodermis in such a manner that the oldest of the light cells are nearest the alimentary tract. My studies on *Photinus consanguineus* show that the reverse is true, for the older cells (as far as position is concerned) are nearest the hypodermis, although from a developmental standpoint they are all of about equal age. Marchal ('11) follows Dubois and regards the photogenic tissue as ectodermal.

Treviranus ('18) seems to be among the first investigators to have decided that the fat-body and light-organ are of the same substance, since he says, p. 102: "Ich finde die leuchtende Substanz dieser Käfer (*Elater noctilucus* und *phosphoreus*) ganz

einerley mit dem Fettkörper derselben." Peters ('41), on page 241, says that, although the fat-body cannot be mistaken for the light-organ, it seems probable that the chief constituent of the latter is fat. Leydig ('57) considers the light-organ an essential part of the adipose tissue.

Von K  lliker ('57) styles the light-organ a nervous apparatus, because the albuminous material of which it is composed is oxidized under the direct influence of the nervous system. He believes, however, with Leydig that the organ is fatty.

Max Schultze ('65) who studied the photogenic organ of *Lampyris splendidula* quite thoroughly, believed that it is related to the fat-body. Emery ('84), who has investigated the organ of *Luciola italica*, considers it of fatty origin. Wieolowjski ('90) thinks that the two layers of the organ differ in origin, the upper or non-luminous being fat-like, the lower ectodermal, since it is composed of certain oenocytes.

Seaman ('91) upholds the derivation of the organ from the fat-body. Wheeler ('92) says: "The resemblance between the fat body and the light organ is so great that I do not doubt their genetic relationship though I have not studied the development."

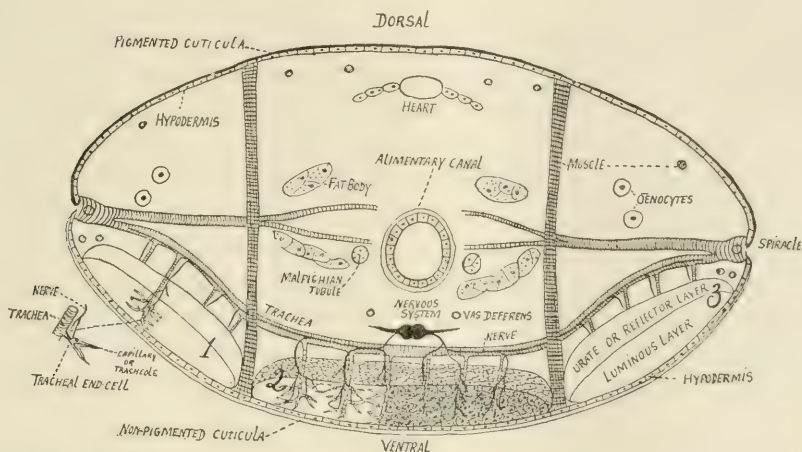
Bongardt ('03), Berlese ('09) Dahlgren and Kepner ('08) believe the photogenic organ to be a derivative of the fat-body, and Vogel ('13) has shown that the light-organ of *Lampyris noctiluca* is derived directly from the fat-body.

Pierantoni ('14) figures in section the light-organ of *Lampyris* and the symbiotic organ of the homopteron, *Aphrophora spumaria*. It must be admitted that there is a striking similarity between the two structures. The small bodies, one or two micra in diameter, observed in the photogenic layer, he believes may be luminous bacteria, which, as in the non-luminous bacteria of the Homoptera, are inherited, since they are transmitted to the egg while it is still in the ovary.

Buchner ('14), in following the opinion of Pierantoni, sees a possible relationship of the light-organ to the specialized fat-body (pseudovitellus) of the Homoptera, and a support of his views of symbiotic mycetoms.

Geipel ('15) gives the structure of the light-organs some study. He believes it possible that all light-organs in the Lampyridae may not be derived from the fat-body.

It is evident that the luminous organs of insects need not be mesodermal in origin, for we have but to consider the very efficient light-organs of the mycetophilid fly of the New Zealand caves.¹



Here, curiously enough, the light-organs are a modified portion of the Malpighian tubules, which of course, are ectodermal (Wheeler and Williams, '15).

STRUCTURE OF THE PHOTOGENIC ORGANS

In all the forms of fireflies (Lampyridæ) which have been studied, as well as the brilliantly luminous elaterid beetle, *Pyrophorus noctiluca*, the photogenic organs, whether of the larva, pupa or adult, present the same general structure. The short description which follows, together with the accompanying diagram, text-figure 1, should give the reader some idea of their makeup. The photogenic organs usually occupy a part or the

¹This fly does not appear to be confined to New Zealand, for Morton ('91) reports what seems to be the same or a very similar insect from the caves of Tasmania.

whole of one or more ventral abdominal segments. Briefly stated, the light-organ is composed of two layers of cells, an inner or non-photogenic (urate or reflector) layer, and an outer luminous or photogenic layer. The inner layer functions as a reflector and is opaque in life and rather translucent when fixed and stained. From the contents of its cells it is also known as the urate layer. The outer or photogenic layer is clear in living, and deeply-staining in fixed material; it owes the latter property largely to the numerous little photogenic granules which sometimes fill its cells. Outwardly the photogenic layer is overlaid by the thin hypodermis, which in this region usually secretes non-pigmented cuticle. Tracheae and nerves penetrate both layers inwardly, but in this respect the photogenic layer is the more richly supplied. The vertical branches from the main tracheal trunks give off many fine tracheae, which terminate as branching capillaries or tracheoles among the photogenic cells. These capillaries are surrounded, at least in part, by cytoplasm, which, together with the basally situated nucleus, constitutes a tracheal end-cell.

DEVELOPMENT OF THE PHOTOGENIC ORGANS IN THE ADULT PHOTINUS CONSANGUINEUS

The development of the photogenic organ of the adult is best studied in the male, for it here occupies the whole ventral portion of segments six and seven of the abdomen. (It is a common error, as pointed out by Vogel, for students of the photogenic organs of the Lampyridae, to designate these organs as occurring on the fifth and sixth segments, etc., when in reality they are situated on the segments immediately following. This can be readily seen in the larva, also by counting the dorsal abdominal segments of the pupa and adult.)

In sections of a larva a day or two after hatching, the fat-body consists of large, often free cells, each containing a single nucleus, and with the protoplasm well vacuolated (fig. 20). They measure about 8-12 micra in diameter, and all appear histologically similar. A larva sixteen days old shows fat cells (fig. 21) which are frequently more or less united into sheets or

layers. The cells are now more irregular in form, and measure about 10–16 micra in diameter. They often contain a few spherical proteid bodies with deeply staining centers. We find that in sections of a second-year larva, the fat cells are much larger and are massed into spherical or subspherical bodies (fig. 24, *FB.*) which occupy a large portion of the body-cavity. These fat-spheres measure about 60–160 micra in diameter, though they vary considerably in size. Their constituent cells have thin and often ill-defined walls, so that the mass may look much like a syncytium. Each cell has a single oval or subspherical nucleus which is surrounded by a small amount of protoplasm. The rest of the cell is filled with blue-staining proteid bodies as well as fewer pale brown ones of the same size. These bodies, which range from 1.16 to 4 micra, are usually spherical, but may also be oblong, or both oblong or crescentic. In addition, very small yellowish-brown granules, resembling coagulated plasma occur in the fat cells. But the fatty substance itself has all been dissolved away in the fixing and staining, and this also appears to be the case with the urates. The fat-body occupies the greater part of the body cavity, where it often appears as a loose and irregular perivisceral sheet. Isolated fat-bodies are also found and seem to be quite similar to those which occur in the larva of *Lampyrus noctiluca*, as figured by Hollande ('09). No breaking up of the fat-spheres, such as is found in pupating larvae and in pupae, was observed.

Situated especially near the insertions of the dorso-ventral oblique muscle bundles are often more or less compact groups of large haemocytes or blood-cells. These may lie in part on the hypodermis, but they are usually separated from it by a small or even a considerable space, and may sometimes occur near the center of the body. Apparently similar cells have been studied in *Apis* and *Vespa*, by Anglas ('00) among others. He does not attribute to them a phagocytic function, but thinks that they may grow at the expense of the degenerating muscle. At any rate they do not appear to be involved in the formation of the photogenic organs. A sagittal section shows clearly that these haemocytes are segmentally arranged.

The fat-spheres which are situated in the ventral portion of segments six and seven of the abdomen, and are one or two rows deep (*A*, figs. 24 and 25), are destined to give rise to the photogenic organ, and may therefore conveniently be called the photogenic fat-spheres. The most ventral of these sometimes lie directly upon the hypodermis. The longitudinal segmental muscles span these and other fat-bodies. None of the photogenic fat-bodies are among those of large size, such as commonly occur in the mid-abdominal region.

The first change observed in the photogenic fat-spheres takes place a short time before the larva enters the pupal stage. In the case especially studied (fig. 24) the larva was quite active and bore no evidence that it was soon to pupate. (It may be said here that pupating larvae and pupae were never found to possess a general effulgence as is noticeable in *Photuris pennsylvanica*, where even freshly emerged adults glowed in the thoracic region. I do not believe that this phenomenon is involved in the formation of the light-organs.) Here the photogenic were found to differ from the other abdominal fat-bodies, first, in taking the stain a little more deeply, second, in having more distinct walls, and third, in having larger nuclei. These changes are in general, fairly evident in a sagittal section.

The next stage observed was the partial envelopment of the photogenic fat-bodies by a layer of yellowish-brown material, staining much like plasma or dermis (figs. 17, 18, and 24). This does not seem to be a secretion from the fat-body as it lies wholly outside the latter. It is at first fairly homogenous, save that there are small black particles along the inner border. It is not improbable that this cap-like layer partially enveloping the fat-body and diminishing in thickness along the edges, is formed from material in the blood, which, together with certain haemocytes, has been attracted to one side of the fat-body by some substance, and has thus come partially to invest it.

The fact that the blood-cells (figs. 17 and 18) are almost always in contact with this investment and not applied elsewhere to the fat-body, would seem to indicate that these bodies exert a certain attraction. Here also coagulated plasma gathers

in the form of yellowish-brown granules. The affinity of phagocytes for the fat-body is a common feature during the metamorphosis of insects. It is claimed that phagocytes may penetrate the fat-body itself, but this does not occur in the case under consideration. It must not be supposed that the photogenic fat-spheres alone are thus affected, nor perhaps that they all have such an investment. On the contrary, partial investment and attraction of haemocytes is far more common in the fat-spheres of the thoracic region and is rather infrequent elsewhere.

Probably the explanation for this is that in the thorax and in the photogenic area, and perhaps also at the caudal extremity, histolysis and histogenesis are much more vigorous than elsewhere. In the head and thorax, at the time of pupation there is a great alteration in the musculature and in the appendages, and some in the nervous system, as well as a great alteration in the body form, but these changes are not so pronounced in the abdominal region, at least as regards the musculature. The photogenic mass with its extensive tracheation must be quickly built up over a large area. It is the direct product of the fat-body, as we shall presently see. The haemocytes, *FB*, associated with these fat-bodies are quite different—usually in size and certainly in appearance—from the segmental blood-cells (figs. 17 and 18). They were not recognized in larvae of the first instar, where the segmental blood-cells were quite marked, but occurred very abundantly in large larvae and in pupae. While the segmental blood-cells are comparatively local, and perhaps rather inactive, the fat-haemocytes, as we may term the others, have a far more scattered distribution throughout the body and are frequently in contact with some tissue. The segmental haemocytes are on the average larger (8–13 micra), more regularly rounded, deeply-staining granular cells, with large nuclei and few or no vacuoles; the fat haemocytes on the other hand, are usually smaller (5.8–12 micra), decidedly vacuolated and therefore of lighter color, with smaller, often irregular nuclei, and though commonly spherical or nearly so, they frequently exhibit amoeboid processes.

In later stages they often mass together, thus somewhat resembling a tissue. They are intimately connected with the fat-body at certain stages and evidently play an important part in the histolysis of muscles, especially those of the thorax. In this region they apply themselves closely to the muscle tissue, though none was found penetrating or ingesting it. Except for their smaller size and the often more irregular shape of their nuclei they much resemble incipient fat-cells. They are naturally more abundant in the pupal stage than at any other time. Blood-cells were very seldom found in the process of division, and when in this condition it was difficult to determine the kind of haemocyte which was under observation.

The partially investing cap as well as the fat haemocytes seem to be instrumental in the breaking up of the fat-body, either into separate cells, as is largely the case in the luminous tissue, or into scattered food particles for the various organs, as was noticeable in the head and thorax. It is noteworthy that in the case of the photogenic fat-spheres the investment is usually on the side next to the alimentary tract, so that when the fat-cells are liberated they are able immediately to migrate to the body wall and there form the photogenic layers. By the inflection of the cap or investment, the fat-sphere is squeezed or constricted at that point till the thin membrane opposite can no longer withstand the pressure, when as a result the fat cells are ejected in masses (fig. 24), and presumably also singly. The pressure exerted often serves greatly to attenuate the nuclei, but no cell division could be detected during the process. Very rarely, and then only in the case of a thoracic fat-sphere, were the cells seen to be liberated through a rupture of the cap. Vogel ('13) has described the breaking up of the fat-body in *Lampyrus noctiluca*, to form the photogenic tissue. The envelope of the fat-body is ruptured, probably through the activity of neighboring leucocytes, and from the old membraneless nuclei of the fat cells arise new ones which are richer in chromatin and possess a distinct nuclear membrane. One might say that the nuclei regenerate, for here there is considerable mitosis, especially on that side of the photogenic fat-bodies which is nearest the hypodermis.)

As the fat cells become more and more expelled, the investment thickens and the number of fat haemocytes often increases to such an extent that in their crowded condition they become noticeably squeezed and attenuated, and form a mass several rows deep (fig. 18), the outer haemocytes being more rounded, the inner closely applied, in a roughly radial fashion to the investment. They frequently contain fine yellowish granules, such as occur in the fat and also constitute the blood plasma.

The ultimate fate of the investment and what may remain of the fat-body within was not definitely ascertained. I often found in sagittal sections through the thorax of pupating larvae, groups of much reduced investments (*I*, fig. 19) circular or U-shape in outline, according to the section, and sometimes containing a network of cytoplasm or a few proteid granules and what seemed to be waste matter. Such investments were still at least partially surrounded by the much vacuolated fat haemocytes whose irregular outlines and frequently attenuated and eccentric nuclei would seem to indicate a degenerating condition. It appears probable that the investments disappear before the adult condition is reached. They were not well defined in the less studied *Photuris* larvae.

The photogenic fat cells which are set free in this manner are large. Their nuclei soon round up considerably. They still contain the large blue-staining proteid bodies so conspicuous in the fat-body proper. The cells (*P*, fig. 25), as yet undifferentiated, migrate to the body wall and apply themselves to the hypodermis, *H*, where they form an uneven layer. Even at this early stage of development in pupating larvae, some of the tracheal branches, arising from the large stigmatic stem invaginated from segments six and seven, push through the photogenic tissue, even to the hypodermis. But the tracheae in the photogenic tissue are not yet differentiated, for they resemble those in other portions of the body (*T*, figs. 24, 25 and 27). In a later stage (fig. 27), it is three or more cells deep, and, though the mass is still rather indefinite, it begins to show a differentiation into two layers. The cells have become denser, containing as they do rather sparse little pale yellow granules and a fine net-

work of cytoplasm. The nuclei, though large, are not so sharply outlined. Furthermore, many of the blue-staining proteid bodies have disappeared. Vogel ('13) in speaking of the contents of the fat cells of *Lampyris* larvæ, mentions these albuminous granules as the chief inclusions. This reserve material he says, much resembles in staining and refraction the yolk spheres in the egg. He describes the transformations of the albuminous inclusions of the fat cells into the photogenic granules as follows, p. 331:

Von Wichtigkeit ist nun das Schicksal dieser Dotterkügelchen in den zur Bildung der Leuchtorgane bestimmten 'Fettzellen.' Dieselben werden nämlichwohl unter Einwirkung von Enzymen—zerstrummert, wobei Vaeuolen (Wasser?) in ihnen auftreten. Die Bruchstücke werden sodann immer kleiner, und schliesslich kommt es dahin, dass wir in den Licht produzierenden Leuchtzellen nur ganz feine eosinophile Granula vorfinden, dass wohl, da sie sich in jedem Leuchtgewebe der Lampyriden finden, den 'Leuchtstoff' repräsentieren dürften.

I have been unable to account for the disappearance of the blue-staining proteid granules (albuminous inclusions) in this manner. It seems to me as if they were first dissolved and used up as food and that the smaller, photogenic inclusions were but the indirect product of the first, just as this reserve material contributes to the formation of various tissues in other parts of the body.) The outer photogenic (peripheral) cells—those which are the seat of luminescence—are already larger than those of the scarcely differentiated upper or reflector layer. Nerves, presumably from the last two abdominal ganglia (as in *Photinus marginellus*, as described by Townsend) and supplying the photogenic tissue, were found at about this stage. They were few in number and quite slender. A single branching nerve extended to a tracheal invagination and could be followed up to the photogenic tissue, which it penetrated. Bongardt ('03) states that in *Lampyris splendidula* he found nerve fibres in the tracheal end-cells. Geipel ('15), who gives good detailed descriptions and illustrations of the light-organs of *Photinus marginellatus* and *Pyrophorus noctiluca*, has found in the former insect a fine nerve penetrating the tracheal end-cells and going

into the nucleus. This is noteworthy, especially from a physiological standpoint, for it indicates that the end-cells are the important mechanism for stimulating the light production.

In the newly-formed pupa a decided advance in development is observed (fig. 28). The two layers can now be clearly separated. The cells of the photogenic layer, *P*, are more spherical, have increased in size and in density of contents, and the nuclei, though larger, are less sharply defined. All of the blue and brown granules, once so abundant, have disappeared, and, owing to a fine knotted network of cytoplasm, the cells have a rather granular aspect. It seems probable that the cells of both layers divide, since the cells of the fat-spheres involved in the formation of the organ seem not to be sufficiently numerous to preclude a later proliferation. The cells of the luminous layer are larger and rather more regularly arranged than those of the non-photogenic layer, *U*, which has resolved itself into several layers of smaller, clearer, more or less flattened cells.

The differentiation of these two layers is further accentuated by the development of the 'tracheal end-cells' which are almost certainly formed from the tracheal epithelium in the lower or photogenic layer. Tracheal end-cells, so named by Max Schulze ('65) who studied exhaustively the light-organs of the European *Lampyrus splendidula*, are cells whose cytoplasm envelop, at least basally, the tracheoles or fine terminal, capillary tubules of the tracheæ. The tracheoles, though chitinous, lack the tænidial thickenings characteristic of tracheæ. Figure 23 illustrates a tracheal end-cell, modified from a sketch by Lund ('11), of the photogenic layer. Holmgren ('95) uses the term 'transition cell' (Übergangszelle) for these end-cells, since he claims that they are not the terminal cells, for there exist other very small and elongate cells distad to them. Townsend ('04) uses Holmgren's term. At any rate it seems reasonably certain that the tracheal end-cells are really terminal in the Lampyridæ. (Tracheal end-cells are not peculiar to the photogenic organs of insects but occur in other tissues, such as the spinning glands of Lepidoptera, in the Malpighian tubules of *Hydrophilus piceus* (Schneider, '02), in the fat-body (Schneider, '02, Thulin, '08),

in the crop of Blattidæ (Petrunkewitsch, '99), etc. Geipel ('15) however was unable to find distinct tracheal end-cells in the luminous elaterid (*Pyrophorus noctiluca*). The end-cells have their cytoplasm continuous with the tracheal epithelium on the one hand, and on the other, spread far along the tracheoles, if they do not entirely envelop the latter.

In general, the tracheæ (*T*, fig. 28) which supply the light-organ, extend transversely from spiracle to spiracle and are connected by lateral branches, well illustrated by Geipel ('15), over the dorsal layer and send down numerous, usually vertical branches through the organ. The epithelium which invests and secretes the chitinous intima of the tracheæ, is, in the non-photogenic layer, as elsewhere without the organ, rather thick, with numerous deeply-staining nuclei which lie parallel to the length of the chitinous tube itself, already being secreted at this stage. In the photogenic layer however, the tracheal epithelium is much thicker (*E*, fig. 28), for here the cells are not parallel to the length of the tube but have their long axis at right angles to it. The nuclei are in the distal portion of the cells. It is in this region that the tracheal end-cells are formed. They occur on the numerous little lateral branches which radiate from the vertical trunks (*EC*, fig. 29), and are close together or contiguous, thus forming a sort of outer cylinder investing the now thin tracheal epithelium on the one hand, and on the other, closely applied to the surrounding photogenic cells. The tracheoles most commonly bifurcate and anastomose, and may often appear in cross section as thick rings between the photogenic cells. Geipel ('15) who has carefully studied the end-cells in *Photinus marginellatus*, concludes that the capillary ends blindly in the middle of the end-cell; that there is no direct connection between this capillary and the processes of the end-cell, that these processes do not anastomose, and finally that the latter are intercellular. Having no osmic acid fixations of this material I am unable to add anything to these disputed points, except that I have found no end-cell processes penetrating the photogenic cells, but have found them to be intercellular. These processes, at least in a number of cases, almost certainly do anastomose,

and this is a common occurrence in end-cells elsewhere than in the light-organ. This can be shown in fresh preparations treated with caustic potash.

In a freshly formed pupa the tracheal end-cells have not yet been differentiated from the thick tracheal epithelium, but cell division seems to be more frequent in the portion of the epithelium immersed in the photogenic layer than elsewhere along the trunk, and it is likely that some of these dividing cells ultimately migrate from their original position along the main trunk, beyond the small branches containing taenidia and secrete the branching tracheoles. The fact that the tracheal epithelium is especially thick in the photogenic layer would seem to strengthen this interpretation. (Geipel found some tracheal end-cells in the urate or upper layer of the light-organ of *Photinus marginellatus*). Still the examination of numerous sections has failed to reveal two sorts of tracheal cells such as occur in the adult.

The photogenic organ, when in the process of formation, frequently suggests a hypodermal origin, for it is then rather structureless and closely applied to the body wall. Indeed some sections show the hypodermis sending up extensions between the cells of the organ. This is perhaps one of the reasons why Dubois ('98) believes that the photogenic organ, at least in *Pyrophorus noctiluca* and in *Lampyrus noctiluca*, is derived by direct proliferation from the underlying hypodermis. It seems to me more logical to suppose that, as in *Photuris* and *Photinus* and *Lampyrus*, the light-organ of all Lampyridae is derived from the fat-body. But unless a complete series of the developmental stages is at hand, it is unsafe to speculate on the origin of the light-organ. A careful examination of the initial stages, I am sure, will convince anyone that the hypodermis has nothing to do with the formation of the light-organ and is distinctly separated from it. The cells of the hypodermis contain no blue-staining proteid granules as do the photogenic cells at first (figs. 25 and 27), and they also appear much too small to give rise to these large cells.

The fat haemocytes were not found to enter or become engulfed in the light-organ, though they could often be seen lying near or against it (*FB*, fig. 28). In this figure the organ is not yet functional, as two or more days in the pupal stage are required before the photogenic power is apparent. By that time the tracheal end-cells are sufficiently developed to supply the necessary amount of oxygen. In the adult firefly the two layers of the organ (*U* and *P*, fig. 29) are so different in appearance as almost to preclude the possibility of a common origin. They are not separated by a membrane and no intermediate conditions between the two layers were observed. The upper or urate layer, *U*, still much resembles adipose tissue, the cell boundaries are well marked and the nuclei distinct, at least in the beginning when the pale granular deposit (urate?) is small. The cells of the lower or photogenic layer, *P*, have become filled with small photogenic granules (0.29–0.58 micra in diameter) thus obscuring the nuclei to a certain extent. These granules are black and are not distinctly spherical; they gradually increase in number and soon render the cells very dark and often obscure their outlines. But the cell peripheries contain no such granules and are therefore much clearer in color. The tracheoles were not found to penetrate the photogenic cells, but the processes were observed to bifurcate and sometimes to anastomose. They are best studied when prepared in caustic potash or osmic acid. Max Schultze ('65) on treating the capillaries of the tracheal end-cells with gold chloride, found them to be hollow to the end, and in life to contain a liquid.

A horizontal section through the photogenic layer shows thick blackish rings forming a sort of network. These rings represent the photogenic cells, separated one from another by pale thin lines. Within each ring is another pale brownish one representing the tracheal end-cells and epithelium, while the central clear space is the vertical trachea. A freshly dissected organ, as seen from the ventral side, shows a somewhat reversed condition, for here the ring of photogenic cells, and probably also the tracheal end-cells, will be found luminous and the enclosed area, the tracheal cylinder, non-luminous.

The two vertical muscle bundles (*M*, fig. 26) which partly divide each photogenic organ, persist, with some changes, from the larva to the imago.

The adult photogenic organs of *Photinus* have been well described and illustrated by Townsend ('04), and Lund ('11), and by Geipel ('15) in Europe.

The larval light-organs begin to deteriorate at least in the pupal stage; the photogenic layer seems to be the first to break up with a disappearance of the nuclei, the layer becoming filled with small black granules or rods. Evidently this is the condition of the photogenic organ (fig. 30) in the adult *Ellychnia corusca*, a lampyrid not held to be luminous in the mature state. Dubois ('98) in speaking of the European *Lampyris noctiluca*, says, p. 321: "Les organes larvaire persisteront après la transformation de la nymphe, soit en insecte mâle, soit en insecte femelle;"

DEVELOPMENT OF THE LARVAL PHOTOGENIC ORGANS IN *PHOTURIS PENNSYLVANICUS*

Owing to the minuteness of this organ, and to the inferior differentiation of the embryonic tissues, the development of the larval light-organs could not be followed with the detail and degree of certitude which the growth of the adult light-organ permitted. It is evident however that as a definite structure, it is formed quite late in the development of the embryo, i.e., when the latter lies in the final curled-up position (fig. 45). It is not improbable that in the earlier stages the light, being diffuse, arises from the yolk granules. Vogel ('13) has made some observations on the development of the photogenic organs in the embryo *Lampyris noctiluca*. He recognises the fatty origin of the organs, and suggests that the source of luminescence is in the yolk, which in its changes, etc., much resembles the proteid inclusions of the fat-body in old larvae. He studied the light-organs in late embryos (18 days incubation) and states that they do not arise from imaginal discs.

It is only late in the embryonic development that the two larval lights are discernible through the egg-shell. Long after

the oenocytes, whose compact and appressed segmental clusters (*OE*, fig. 51) much suggest light-organs, have migrated from the body wall into the body cavity, and the fat-body and haemocytes have become differentiated, but before the cuticula and hairs have appeared, the photogenic organ (*O*, fig. 15) is discernible as a compact mass of rather large, distinct and well-nucleated cells, lying on each side in the eighth abdominal segment, between the anterior end of the segment and its tracheal invagination. The organ is closely applied to the hypodermis, but is nevertheless distinct, though the hypodermis is quite thin where it is in contact with the organ. This fact might suggest that the hypodermis gave rise to the light-organ, but the former is of variable thickness and its cells are quite unlike the nearest cells in the light-organ. These moreover, do not appear to be the youngest cells, as should be the case were they of hypodermal origin, as Dubois affirms of *Lampyrus noctiluca*. On the other hand, the inner cells, i.e., those nearest the fat-body, which appears almost as a syncytial mass, greatly resemble those fat cells (*A*, fig. 15) with which they are in contact. They are more vacuolated here and this suggests their fatty origin. The whole cell-mass is approximately spherical and measures about 17 by 21 micra. The cells for the most part are already differentiated from the fat-body, as their protoplasm is denser and contains small yellowish-brown granules. The cells are also smaller in size. No tracheae as yet penetrate the organ, nor could nerves be found in connection with it. A large cylinder of tracheal epithelium *T*, is near, but has as yet secreted very little or no chitin. The stage of development above described could be found in but a single specimen. The cells indicated a spiral arrangement and gave no indication of the two layers. No blue-staining proteids occurred either in the fat cells or in the light-organ; the yolk mass probably furnished much of the food material.

A considerably later stage of development (fig. 14) is to be found in the well-chitinized larva, with hair, spines and appendages completely or nearly completely developed. Such larvae are capable of moving within the egg-shell, and their photogenic organs appear through the latter as two points of light. The

tracheae, *T*, have already been secreted by the flattened epithelial cells.

The light-organ, which is now quite free from the underlying hypodermis, has assumed a slightly oblong form, and is held in position at several points by membranous strands resembling connective tissue. An outer, somewhat paler layer of cells, *U*, is now obscurely visible, and appears to surround the entire, denser inner mass, *P*, the true photogenic tissue. This seems to be more reticulate than granulate, though in neither zone are the cells themselves clearly discernible, as in the earlier stage, though their nuclei are regular and distinct. It is probable that by this time the organ is functional. It is supplied by a trachea and perhaps a nerve. The fat cells (*A*, fig. 14) are large and vacuolated, with somewhat collapsed nuclei, and contain no blue granules such as are found later. They sometimes lie free in the body cavity like haemocytes, but may also be united into masses. The fat-extruding or -squeezing process through the agency of a partial encasement, described in old larvae and in pupae of *Photinus consanguineus*, was not observed to take place in the embryo, but such a process seems unnecessary here, since the fat-body has many free cells and is otherwise of a much looser structure than in the later stages. The organ now measures about 24 by 34 micra.

In the second-year larva the organ (fig. 16) is about 577 micra in its longer and 451 micra in its shorter diameter, and is broader than high. The whole is enclosed in a delicate, non-cellular membrane by which it is secured to the attenuated hypodermis and there flattened. The hypodermis is now very much thinner than the cuticula. The two layers are well differentiated, but it is evident that the organ is by no means as definitely and highly developed as in the adult. While the light-organ is under control of the larva, in that it can be extinguished and made to glow, this operation is incomparably slower and therefore less perfect than in the adult, where the stimuli are apparently to a large extent transmitted through the optic nerve, a structure which is of course also better developed in the adult.

The inner layer (or rather, mass) is surrounded dorsally and laterally by the thinner outer sheet, and is ventrally applied to the hypodermis, its cells are ill-defined, with distinct nuclei, and the cytoplasm contains numerous, very fine black granulations, about 0.29 to 0.58 micra, which are thus of the same size as those in the adult light-organ. This photogenic mass is quite distinctly separated from the rather clear, more feebly staining outer sheet, which is one or two cells in thickness, the outer being tall and columnar and radially arranged around the center of the organ. These cells of the outer (reflector) layer are all well-defined and filled with small pale granules. At least one large tracheal branch and one fair-sized nerve (*T* and *N*, fig. 13, *Photinus consanguineus*) enter the outer and penetrate the inner mass by a well-defined pore. The nerve is a branch from the most ventral of the three pairs which proceed from the last (eighth) abdominal ganglion. The latter is situated in the fifth abdominal segment. The trachea involved branched considerably within the organ, and terminated as tracheoles (fig. 22) of much slenderer proportions than those in the adult light-organs (fig. 23). No end-cells were found. The capillaries or tracheoles extend throughout the photogenic mass in an intricate and irregular fashion, so that the tracheal cylinders are not evident.

The larval light-organs are essentially similar in *Photinus* and *Photuris*. When freshly dissected the dorsal layer is dead white, and the lower a sort of very pale yellowish green or gray and semi-translucent.

PHYSIOLOGY OF THE PHOTOGENIC ORGANS

It is needless to say the study of photogenic organs from a physiological standpoint has proved to be a subject of unusual interest, especially since it suggests, even to the casual observer, the possibility, however remote, of using the luminous powers in the organic world. The fact that "no sensible heat accompanies the fireflies' light," and that "nature produces this cheapest light at about 1/400 part of the cost of the energy which is expended in the candle flame" (Langley and Very, '90), opens a very inviting field in both pure and applied science.

The photogenic organs in fireflies (Lampyridae) and the neotropical elaters of the genus *Pyrophorus* are perhaps the most efficient and highly specialized of all photogenic tissues, and for this reason, as well as because of their general abundance they have for years been the favorite objects of study in this field.

Förster ('82) advanced the hypothesis that the luminous material of the Lampyridae was phosphorus dissolved in the animal fluids. He found that the Lampyridae shone more intensely in pure oxygen than in air.

According to Lindemann ('63), Treviranus explained the luminescence in these insects by stating that the fat-body absorbed phosphorus. Treviranus ('18) says, p. 109: "Der Einfluss des Thiers auf das Leuchten geschieht mittelbar, durch das Athemhören," and further: "Das Ausstrohmen des Lichts hangt daher von der Wilkuhr des Kifers ab." Treviranus found no nerves going to the luminescent mass.

Another of the older explanations of the phenomenon of light production, is, according to Lindemann, that held in the beginning of the nineteenth century, by Monti, Carradori and Beccaria, who thought that fireflies were light-absorbers (thus comparable to certain minerals) in having the power to take in the sunlight, and in the night, to give it out again.

Michael Faraday ('14) made some interesting observations on the luminescence of the firefly and glow-worm. He was of the opinion that the light "has a dependence on the respiration" . . . and that the light-giving power seemed to depend "more on the chemical nature of the substance than upon the vital powers of the animal." He found no sensible heat accompanying this production of light.

From the middle of the last century, with the advance in chemical and physiological research and the perfection of the microscope, the phosphorus theory was soon abandoned, to be replaced to a large extent by one of the oxidation of an albuminous substance, and this with some modifications is the general view of the present day.

As early as 1857, von Kölliker was able to analyze the two component layers of the photogenic organ, the inner or non-

luminous layer of opaque (weisse) cells containing birefractive crystals of ammonium urate, and the outer (blasse) clear cell layer containing an albuminous substance. Von K  lliker found both nerves and tracheae branching into the lower (outer) layer. He states that the luminescence is the accompaniment of the oxidation of the luminous material and that this oxidation is under the direct influence of the nervous system.

Lindemann ('63) states that the so-called phosphorescent spheres (Phosphorkugeln) are found in the fat-body of all insects, where they never shine, though their chemical composition is the same, and concludes that these bodies are not the real seat of luminescence.

Schultze ('65), and Owsjannikow ('68) made important physiological contributions, the former making good use of osmic acid for the finer study of the tracheal end-cells.

Heinemann ('86), among others, recognizes that moisture and oxygen are essential for the light-production and thinks that the light is not ascribable to the living protoplasm itself but to one of its products, which shines on contact with oxygen brought in by the tracheal capillaries. The light process goes hand in hand with the production of a brownish-yellow material which is diffusely distributed in the cell material.

Seaman ('91) says that the phosphorescence in the firefly coincides with the inspiration and exhalation and not with the movements of the heart. He devotes much space to the chemistry and physiology of the light-organs. He says, p. 143: "It is by means of the muscles that act as intermediaries that the nerves regulate the photogenic function." Respiration, he says is only an indirect control.

Dubois ('98), in his extensive studies on animal luminescence, holds views quite different from the men of his day and of the present time who have studied the phenomenon. He states that the cells of the upper or non-photogenic layer are nothing more than the cells of the luminous zone which have undergone histolysis. He does not attribute to oxygen the primary role but only the capacity it plays in other tissues (reducing agent), and holds that the luminescence is caused by the conflict of two sub-

stances, the first he calls luciferase, resembling an enzyme and resulting from the granular degeneration of bioproteon in the photogenic cells, the second substance he calls luciferine, which he obtained in an impure state (and had not then analyzed) he thinks occurs in the blood throughout the body, and considers it to be non-living material, inasmuch as it can withstand temperatures incompatible with life, with the addition of oxygen and water. (Later ('11) Dubois abandoned these two terms, which he states were provisional and which gave rise to disputes of a compromising nature.) As a passageway for the blood carrying the luciferine to the photogenic organ he figures large spaces or cracks in the latter. The existence of such spaces seems improbable, as they do not appear to have been observed by any of the subsequent students. Lund ('11) believes that the spaces are "nothing but the region of the vertical cylinders and tracheae which sometimes do not stain readily." Dubois bases his conclusions largely on the luminous bivalve mollusc, *Pholas dactylus* L.

Wielowiejski ('82) thought that the tracheal end-cells in the luminous layer, as well as their tracheal matrix were analogous to the red blood-corpuscles of vertebrates. The oxygen of the air going into the tracheal capillaries would be absorbed and given over to the proper photogenic cell. The nervous system would excite the parenchyma (photogenic) cells to secrete, and photogeny would be a process of simple oxidation.

Bongardt ('03) repeated the various experiments of Dubois but obtained somewhat different results. He holds that Dubois' experiments were often of too short duration, and perhaps the gases used too impure to give trustworthy results. Bongardt made an extensive study of the physiology and the finer structures of the photogenic organ. He found many nerve processes entering the photogenic layer, following the tracheae, but unlike Owsjannikow, never found nerves penetrating the cells themselves, although he did find them penetrating the tracheal end-cells. He considers the luminescence in the Lampyridae to be a secondary phenomenon.

Kuhnt ('07) suggests that possibly the light produced by Lampyridae is bacterial.

Dahlgren and Kepner ('08) say with reference to the tissues of photogenesis that: "The power is probably a specialization of the same or similar processes to those that produce heat, motion, and electricity." They do not accept the theories of Dubois and do not consider the luciferine of the blood, but oxygen, the reducing agent in photogeny. Of the luciferase of Dubois they say: "It can be seen in sections and teased cells as a collection of granules that stain very readily and retain the stain with great tenacity."

Lund ('11) studied the light-organs in fireflies from a structural and physiological point of view. He is of the opinion that the process of photogenesis is dependent upon the presence of a substance, probably of the nature of a reductase, in the presence of water and oxygen, and that it is probably an oxidation process. He determined that the dorsal layer of the photogenic organ became "the repositories for the waste product" (of the lower layer) and that

From the facts concerning the relation, in amount, of the granular deposit in the dorsal and ventral layers it is evident that we are to consider the granules of the photogenic cells as at least one if not the main source from which the crystalline (urate) deposit is derived. . . . From a study of the dried photogenic material we at once see, as others have long ago pointed out, that the immediate process of light production is not dependent upon the protoplasm of the cell but upon the interactions between formed substances.

Harvey ('14) studied the chemical nature of the luminous material of the firefly and believes that the phosphorescence is due to the oxidation of some substance formed in the cells of the animal. He says in part: "I can state definitely that the 'luciferine' of the common firefly is not a true fat or any fat-like body such as lecithin. . . . The material is therefore insoluble in fat solvents. . . . It is most likely a protein but belongs among the proteins insoluble in water."

Pierantoni ('14), and Buchner ('14), think that the photogenic organ may be a luminous bacterial structure. Buchner states that in bacterial symbiosis in Coleoptera thus far observed, the infection is through the mouth of the insect. (Dubois has made numerous attempts to determine if possible whether the photo-

genic granules were bacteria, by trying to make cultures of these granules in a number of different media. All his results were negative.)

Geipel ('15) who has published perhaps the best account of the structure of the photogenic organs in beetles, agrees with Bongardt when he says that the nerves to the photogenic layer terminate in the tracheal end-cells and not in the photogenic cells. Thus it would seem that the oxidation process for photogenesis is activated through the agency of these end-cells.

It seems clear that while the photogenic organ in the Lampyridæ is derived from adipose tissue, the luminous substance itself is not fat-like, but is probably a protein contained in modified fat cells (the luminous layer) in the form of minute dark-staining granules. Such granules evidently occur in all true photogenic tissue.

Almost numberless experiments have been performed with fireflies to determine if possible the manner as well as the purpose of the light production. An old and important discovery is that when the photogenic organ when dissected out and dried, it will, months thereafter, shine in the presence of air and moisture, showing that these substances are essential to luminescence, and that the process may be independent of living protoplasm. But the very fact that it is produced by protoplasm (plus oxidation) which is not generally admitted to be a merely physico-chemical combination, would necessarily entail a vital activity of some sort. That the luminescence is controlled directly by the nervous system and not immediately through muscular activity (abdominal respiratory movements) seems clear from experiment as well as from the fact that glandular structures in general (including the electric organs of fishes) are dependent for their activity upon nerve control. Luminous organs may also respond locally to mechanical stimuli. It is easy to determine that the respiratory movements do not correspond to the periodicity of the light, and as Lund and others have shown, there is no special musculature for the photogenic tissue. Nor does it seem probable that the light is controlled by the flow of blood, as claimed by Dubois. This could hardly account for the rapid and well-

defined flashes of light and would necessitate the control by a well developed system of muscles of large passages (which do not exist) in the light-organ. Lund severed the nerve cord of *Photuris* and found that the insect lost control of the photogenic organ; at most a faint residual glow persisting after the operation. In a similar experiment on the larva of this insect I found that the light would quickly lose its brilliancy and finally disappear.

That the luminous substance is at certain periods also in a diffuse condition, i.e., not confined to a light-organ, is evident if one examine eggs which are not far developed. This diffuse condition is still better shown in pupating larvae and in pupae of *Photuris pennsylvanica*, and it even prevails in freshly hatched adults, where it is more clearly visible in non-pigmented areas, as at the sides of the pronotum. Effulgent pupae were cut in three pieces to make sure that the glow was general. Dissections showed points of light (granules) such as occur in the true photogenic layer. This diffuse light was more pronounced in the head and thorax, and did not supplant the true photogenic organs at any time. The dorsal vessel had no effulgence and it seems probable that the luminous granules are distributed at such times also in the yellowish-white fat-body which pervades the insect. Such a glow seems, so to speak, to be a necessary accompaniment of the rapid metabolism which takes place at this stage, and subsides as the development becomes the more complete.

Dubois (1886, p. 100) says, that at the moment of metamorphosis in the larva of *Pyrophorus* and in the pupa of *Lamproyris* (when histolysis is much more rapid than at any other time) a diffuse glow spreads over the body. No such luminous phenomenon was observed in the larva and pupa of *Photinus*. It does not seem probable that these diffused photogenic granules finally collect in the light-organs, as do for example the bacteria in the mycetoms of Homoptera, but that they are used up in situ, the granules of the photogenic layer being formed in the cells of that organ itself.

The spectrum of the firefly has been studied by a number of men, among whom are Young ('70), Langley and Very ('90), and Dubois ('98). To quote Young:

The spectrum given by the light of the common firefly of New Hampshire (*Photinus*) is perfectly continuous, without trace of either bright or dark. It extends from a little above Fraunhofer's line C, in the scarlet, to about F, in the blue, gradually fading out at the extremities precisely this portion of the spectrum is composed of rays, which, while they more powerfully than any others affect the organs of vision, produce hardly any thermal or actinic effect.

By dissecting out the light-organs in living specimens and then observing the same through the spectroscope a weak spectrum was obtained; by adding hydrogen peroxide to the photogenic organs the light, apparently unchanged in color, became more intense and steadier.

The purpose of the photogenic organs in insects has been much studied. McDermott and Mast, among others, consider the luminescence in the Lampyridae to be a secondary sexual character. Others offer the explanation that they furnish protection to the insect in their warning or intermittent light emissions. Olivier has made some interesting observations upon the secondary sexual characters of these insects and shows that there is a definite relation between the development of the photogenic organs, the eyes, and the antennae. It is the combination of these, and perhaps other organs which constitute a composite sexual character.

Inasmuch as the larvae of many Lampyridae at least, are gregarious, in that they inhabit defined areas, it is possible that in this case, luminescence may assist in the preservation of the colony.

The adults of luminous Lampyridae are known to have a rather well-marked periodicity in exercising their photogenic powers, shining normally in the evening and not being long deceived when placed during the day, in darkness. But *Photinus pyralis* has been known to fly and to scintillate in the darkness caused by the approach of a heavy thunder shower (see Laurent, Ent. News, XXV, p. 334, 1914). But the larva of *Photuris* seem to

have this character of periodicity less pronounced than the adult. Some of these larvae on being placed at 11 a.m. into a dark room, emerged from their hiding places, and while not glowing with their usual brilliancy, walked about and fed as if the evening were at hand.

NOTES ON THE EMBRYOLOGY OF PHOTURIS PENNSYLVANICUS

The egg (fig. 31) is subspherical, pale lemon yellow, and measures about 784 by 677 micra in diameters. It has three envelopes; first, a thin outer membrane which encloses many fine oil-like drops and gives the surface a granular aspect; second, the chorion, the stoutest and firmest of the envelopes; and third, the vitelline membrane, a secretion of the egg itself. The dorsal and ventral sides of the egg appear similar, but the longer axis evidently marks the anterior and posterior poles. I could find but a single micropyle, and this was to one side of the presumably anterior pole.

I have no stage which shows the formation of the blastoderm, or peripheral cell layer. Figure 32 is a section through an early blastoderm stage. No cell walls are visible, the cells being flattened against the vitelline membrane on the one hand, and on the other they bulge inwardly. The vitellophags or yolk cells (*V*, fig. 32, and fig. 42) are considerably larger than the blastoderm cells, and are often amoeboid or stellate. They are not very abundant in the stage figured, but later they increase in number, and doubtless, through the agency of protoplasmic processes, form an extensive network.

Embryonic envelopes. The amnion and the serosa appear before there is any indication of a gastrula. This is clear from figure 33, where the amnion, *A*, is thicker than the more flattened serosa, *B*, and merges almost imperceptibly into the somewhat thicker germ-band proper, *G*. A dorsal view of the embryo at this stage is shown in figure 34. It is about 245 micra long and is thickly almond-shaped, depressed, the broad blunt end being the cephalic one. The paler diamond-shaped area represents the point of contact between the amnion and the serosa.

A few cells may protrude into the amniotic cavity (fig. 33), at the point where the closure of the folds is effected. The cells of the blastoderm (the peripheral cells shortly separated from the embryo and commonly termed the serosa) have fairly distinct walls and are fusiform-flattened. The yolk, *Y*, which stains very deeply, is massed into subspherical bodies about 0.6 to 6 micra in diameter, and the vitellophags are much less evident, though now probably more numerous. Yolk granules may occur in the cells of the germ band as well as in the blastoderm.

Before gastrulation, the embryo both lengthens and broadens considerably, though its dorso-ventral thickness increases but little. Heretofore quite straight in its long axis, it curves dorsally and thus partially embraces the yolk-mass. It appears constricted before its middle, because the anterior portion has been broadened and furrowed to form the procephalic lobes (figs. 35 and 36). It is now about 263 micra in its straight length. It has become separated from the serosa, and, sinking towards the center of the egg, becomes a germ band of the immersed type. (Quite late in development, the amnion and the serosa come in contact in a comparatively small area in front of the mouth, (fig. 52)). Here the amnion and the serosa are separated from each other by an intermediary mass of yolk. This condition is represented in a later stage in figures 51 and 52. It seems that the immersed type of germ band is unusual in the Coleoptera. According to Korschelt and Heider ('99) the germ band of Coleoptera ". . . is superficial and is grown over by the forward extension of a caudal fold and paired cephalic folds. . . . The posterior end of the germ band on the contrary develops entirely according to the invaginated type." But later, by shifting, the germ band becomes totally superficial. Such a condition is found in *Hydrophilus* and *Lina* (Graber), *Chrysomela* (Strindberg), and probably in others.

Figure 37 shows a later stage of development. Segmentation is not yet apparent, but the embryo has greatly increased its size and curvature, for it now measures 390 micra in its full length. The cephalic lobes, *P*, are wide and separated anteriorly by a deep cleft. The amnion, *A*, forms a stout envelope,

and is especially thick at the caudal extremity, where it is often two cells in thickness. It merges gradually on each side into the germ band. The latter has as yet scarcely reflexed its borders towards the yolk-mass, but the gastrula, which is of an invaginated type, has already closed its lumen or central cavity, although it is still recognizable as a groove over most of the germ band. The invaginated portion seems to consist altogether of homogeneous mesoderm.

The next stage observed was the beginning of metamerization. Two small lobes (*L*, fig. 38) on each side of the median line represent the labrum, a clear central space the mouth, while a pair of subangular projections, *F*, the antennae. The latter already bear indications of segmentation and constitute the posterior portion of the cephalic lobes. All of the remaining segments have rather uniform proportions, and their appendages, including the first abdominal, are inconspicuous swellings. The amnion has become a thin sheet and remains such from now on. By the dorsal flexure of the sides of the embryo, the point of origin of the amnion has been moved towards the dorsum, where it is sharply marked off from the thick ectoderm (compare figs. 46 and 48). The serosa, which lines the interior periphery of the egg, is, like the amnion, a flattened sheet, but its cells are far larger and less attenuated than those which compose the amnion (*S* and *A*, figs. 51 and 52).

A slightly older embryo is shown in figure 39, in lateral view, and in figure 40, straightened out, in ventral view. The body is stouter, somewhat fusiform, and its segmentation is well defined. The cephalic lobes are further subdivided, the paired lobes of the labrum being quite large, the antennal rudiments more elongate and cylindrical, and the mouth-parts more distinct, especially the first maxillae. The legs, *LG*, and the pleuropods, *IA*, of the first abdominal segment are conspicuous enough, though the remaining abdominal appendages are decidedly obscure. The caudal end of the abdomen is bilobed. The embryo is bent backwards nearly into a circle. Cross sections of this stage (figs. 47 and 48) show much advance over that represented in figure 46. The sides of the body are well reflexed and the

neural groove, *G*, is quite deep, or nearly closed. The lower layer, *M*, has proliferated so as to constitute almost the entire dorsal portion of the embryo. Development has, as usual, progressed more rapidly at the anterior than at the posterior end (compare figs. 47 and 48). Figure 48, a cross section through the thoracic region, shows several large cells, *NB*, lodged in the ectoderm, on either side of the median line. These are the neuroblasts which are just beginning to give rise to ganglionic cells. They are not nearly so symmetrically arranged as are those of the orthopteron *Xiphidium*, studied by Wheeler ('90). A dividing cell, which protrudes from the ventral side of the embryo, strongly suggests the beginning of an appendage (*X*, fig. 48). By a process of splitting, the mesoderm, *M*, has given rise to the primitive coelom, *IC*. Its ventral are thicker than its dorsal walls. More posteriorly (fig. 47), neither the neuroblasts nor the primitive coelom are as yet discernible.

A stage intermediate between those of figures 39 and 41, is shown in cross section in figure 49 (abdomen). The sides of the body are further reflexed, the ganglia are marked swellings and contain a large number of ganglion cells which are lodged in a paler fibrillar matrix. The legs, *LG*, are not yet invaded to their extremities by mesodermal elements. The primitive coelom, *IC*, is no longer slit-like and appears in horizontal section as a pair of lateral sacs in each segment. Such sacs were found in the mandibular and all the following somites. The permanent body cavity or schizocoele (*C*, figs. 49, 50 and 51), is formed partly by the withdrawal of the inner walls of the coelomic sacs from the yolk-mass, and partly by the breaking down of the walls of these sacs. In the schizocoele are a few large free cells, with vacuolated protoplasm and large nuclei. These are probably haemocytes, *H*, which seem to have become separated from the inner, upper wall of the coelomic sacs, the splanchnic mesoderm, which early breaks down at this point. Just dorsal to the ganglionic swelling are several large pericardial cells, *PC*, which stain more deeply yellow. Like the haemocytes, they appear to be derived from the splanchnic mesoderm.

Figure 52 is a sagittal section of a more advanced embryo, such as is illustrated in figure 41. The yolk-mass is being

rapidly absorbed, and, with the exception of a comparatively small area at the cephalic end, is interposed between the amnion and the serosa. The central yolk-mass, *Y*, is becoming enveloped by a thin entodermal sheet *ENT*. The latter encloses a muscular layer on the ventral side of the yolk. The stomodaeum and the proctodaeum (*ST* and *PR*, fig. 52), are thick-walled tubes of columnar cells which are bounded externally by a muscular layer. A shelf-like extension near the inner end of the proctodaeum evidently represents a valve. A thin sheet of entoderm shuts off these two invaginations from the forming mid-gut. The ectoderm, in general quite thick, thins out intersegmentally and where it passes over into the amnion. The pleuropodia, *IP*, have reached their full size, and have evidently discharged most of the secretion. The central nervous system is compact and proportionally of great size so that the connectives are very short. The punksubstanz or central fibrous material, *F*, occupies the more dorsal inner part of the chain and is enveloped by ectodermal epithelium, the inner neurilemma *IN*, (innere Nervenscheide, neuroglia), while the chain itself is ensheathed by a thinner though more distinct layer, the outer neurilemma or perineurium *ON*, also probably ectodermal. Both of these envelopes are figured by Graber ('89) in *Stenobothrus*, and by Wheeler ('89) in *Doryphora*. The remainder of the body cavity is to a great extent, occupied by irregular sheets of fairly well differentiated adipose tissue, by blood-cells, and by the large rounded yellow pericardial cells. The gonads, *GN*, a compact group of large cells, lie in the dorsal part of the fourth abdominal segment. What I take to be the 'suboesophageal body' of Wheeler is represented by a conspicuous bunch of large yellowish cells, *SG*, which partly invests the posterior end of the stomodaeum, on the ventral side. Except for the more vacuolated and granular contents, these cells, closely resemble the pericardial cells. Of the suboesophageal body, Wheeler ('92) says in part:

It is apparently the earliest organ to be differentiated from the walls of the coelomic sacs. Its cells, at first wedge-shaped, gradually increase in size, become rounded and highly vacuolated and resemble the fat-

body elements, from which they may, nevertheless always be distinguished by their peculiar yellow tint. I have traced the organ which is a definite circumscribed structure, and which I call for the present, the suboesophageal body, through the embryo into the larva, where it disintegrates and finally disappears. I regard it therefore as a truly embryonic and early larval structure, quite distinct, at least physiologically, from the fat body. Its function is very doubtful.

He suggests that it may be nephridial. Both the pericardial cells and the suboesophageal body are formed before the fat-body is well differentiated. The pericardial cells are now the largest cells in the body.

Figure 51 is a cross section through the abdomen of an embryo of about the same age as that illustrated in sagittal section. The ectoderm is quite thick except over the median portion of the ganglion. On each side, closely appressed to the ventral ectoderm is a subspherical mass of pale cells, the oenocytes, *OE*. These have been shown by Wheeler ('92) to be of ectodermal origin. At least six such segmental clusters were found in the abdomen of the *Photuris* embryo. The hypodermis is somewhat thinner where the oenocytes are applied, as if they had been detached at the expense of this epithelium. The oenocytes, compactly massed, with the cell boundaries indistinct, the nuclei dark and reticulated, and the cytoplasm pale and vacuolated, little resemble the large free oenocytes of later life, where, with the exception of the eggs, they become the largest cells of the body. However, they do not surpass the pericardial cells for some time. They become spherical, migrate to the interior with the invaginating tracheae and mingle with some large reticulated fat cells. Oenocytes also occur in the adult beetle where they are often wedged in between the fat-body and have of course lost their segmental arrangement. The gonads, *GN*, are a compact group of large cells lodged in the splanchnic mesoderm, above and lateral of the paler fat-masses, *FB*.

The large arcuate embryo (fig. 41) must reverse its position, i.e., become coiled. The amnion is ruptured in this process. Figures 43 and 44 represent a *Photinus* embryo, which has become very stout and broad, undergoing this involution. The operation begins at the anterior end, as the caudal portion is

seen to be almost in its original arcuate position. The yolk-mass is becoming constantly smaller in proportion to the size of the embryo.

A *Photuris* embryo almost ready to hatch is shown in figure 45, lying in the final curled position. It now measures about 600 micra in the diameter of its curled position. Several days before emergence it is capable of slow movement, which is plainly visible, for the luminous organ, now functional, will be found to change its position.

The fate of the embryonic envelopes was not determined, no dorsal tube (the remnants of the serosa) was found, though it is probable that both the amnion and the serosa are eventually absorbed into the dorsal part of the body.

The mesenteron or midgut is much distended with the greater part of the deeply-staining yolk material, so that the freshly-hatched larva begins life with a full stomach.

THE PLEUPODS OR FIRST ABDOMINAL APPENDAGES IN THE EMBRYOS OF *PHOTURIS* AND *PHOTINUS*

In both *Photuris* and *Photinus*, the pleuopods, so named by Wheeler ('90) are very conspicuous by reason of their size and structure. In neither genus was the organ observed in the first stages of development. In a *Photuris* embryo about one-third developed and before the enveloping amnion has been ruptured (fig. 39), the appendage, *IA*, is a knob-like process with a sort, stout peduncle, and, like the thoracic legs, with its axis somewhat inclined caudally. It is a little less in diameter than the length of the segment from which it originates. In a sagittal section through the organ, its cells, which are of course hypodermal, are in large measure, strongly differentiated in that they are columnar, with larger, very elongate nuclei, three or more times as long as wide, the whole forming a somewhat convex layer or disc (fig. 54). At the distal extremity of the pleuopod there is already much granular secretion, which is evidently extra cellular and held in situ by the thin enveloping membrane, *C*.

A somewhat later embryo (fig. 41) shows the organ *IA*, considerably enlarged, its disc broader and flatter and the neck

proportionally shorter. The cells are four to six times as long as wide and form a flat disc. The nuclei occupy an approximately central position in the cells which may already be vacuolated just below the nuclei, but elsewhere well filled with a granular secretion which largely obscures the cell boundaries. The thin capping membrane is here frequently separated from the apices of the cells. Fat cells largely occupy the base of the peduncle.

A later stage, such as occurs in larvae nearly ready to hatch (figs. 45 and 55), shows that the gland has already largely performed its function. The cap *C*, may be depressed, irregular or wavy, the gland cells, *G*, now well below and separated from the cap, form a concave disc or crater, and seem almost to have exhausted the secretion, for very little of it is in evidence. The now spherical or subspherical nuclei, of the same height in each cell, strongly augment the crater-like appearance of the layer. Each cell has the walls closely applied to those of its neighbors, but the dividing line, *B*, between is usually discernible. Extending from just above the nucleus is the large conspicuous gland duct, *D*, flaring somewhat distally and often saccate at the base, so that the whole glandular structure, distally, has a papillose aspect. The basal portion of each cell, i.e., immediately below the nucleus, is marked by a large space or vacuole, *V*, beyond which are smaller indefinite vacuoles and protoplasmic strands. No muscle was found to connect with the organ, and in but one case what seemed to be a duct, leading from the distal portion of the gland into the body cavity, could be made out. The unmodified hypodermis, *H*, extends well up the sides of the cup where it passes off rather abruptly into the outer gland-cells. Viewed from above, the tips of the gland-cells form a network, the ducts constituting the circular or subangular spaces, the meshes, the cell walls.

It is quite evident that these large gland-cells are hypodermal and modified by elongation, etc. and from the jointed appendage, which, at first bilaterally symmetrical, becomes perfectly radial and extends somewhat pleurally of the thoracic appendages.

A larva quite near hatching shows the gland in the process of collapsing and of sinking into the body cavity. A freshly hatched larva, i.e., one a day old, has already lost the first abdominal appendages, remnants of which can be found in the body cavity. (According to Patten the pleuropods in *Acillus* are absorbed, while in the June-beetle, *Melolontha vulgaris*, they are probably pushed off (Graber). In other cases they seem to be in part cast off and in part absorbed). The gland-cell nuclei are now clearer, show scattered large pieces of chromatin; the cells themselves are breaking down, and the whole is forming a rather deep U-shaped mass. Large phagocytes occur in the vicinity; the cuticle and unmodified hypodermal cells are present above the organ, so that it is probable that they have spread over and covered the void made by the retracted gland-cells.

The pleuropod of *Photinus* is somewhat different morphologically from that of *Photuris*. It is more convex and elongate, the cells form a convex instead of a later, flat disc, their nuclei are distal, and correlated with this is the apparently internal basal secretion instead of the largely distal one as occurs in the pleuropod of *Photuris*. A conspicuous receptacle of non-glandular cells, *E*, calling to mind the cup of an acorn, surrounds and constitutes the basal portion of the pleuropod.

The remaining abdominal segments in either genus bear very small and inconspicuous appendages.

Korschelt ('12) describes and figures in several stages, the pleuropodia of *Dytiscus marginalis*. Evidently their development and their degeneration much resemble that in *Photuris*, in that they at first bear some resemblance to a thoracic leg, later become somewhat mushroom-shaped, with longer nuclei and a glandular character, and finally cave in and sink into the body shortly before eclosion. Korschelt refers to the pleuropodia as 'Drüsenorgane.'

The pleuropods were first noticed by Rathke, in 1844, in the mole cricket, *Grylotalpa*. They do not appear to be of general occurrence among hexapod embryos. They are here (as in the Orthoptera and some Hemiptera) much larger than the following abdominal appendages. Many transitional forms occur. In

the Lepidoptera and Hymenoptera, according to Korschelt and Heider ('99) the limb rudiments of the first abdominal segments are, in some cases less developed than those of the other segments, and in no case do they attain a greater development. According to Graber, they are most leg-like in Mantis and developed into very large vascular sacs in Melolontha. In other cases they are mushroom-shaped, stalked cups, or they may be sunk below the surface of the body, i.e., invaginated (Hemiptera). (Dr. Wheeler thinks that the immersed condition of the hemipterous pleuropodia represents a late stage where such a condition is to be expected.)

The pleuropodia have been regarded as transitory respiratory, sensory, supporting, and glandular structures. The last view though indefinite, appears the most logical, since in most instances they are distinctly glandular in character. Wheeler thinks that the pleuropodia in ancestral insects may have functioned as odoriferous glands. Since they are so well developed in many cases, it is reasonable to suppose that they must be of some importance. In *Photuris* and *Photinus* embryos, stained in toto with borax carmine, the pleuropodia took the stain more deeply than the general body wall and more conspicuously than the other appendages, thus showing the more permeable character of their membrane.

SUMMARY

1. The two species of fireflies, *Photinus consanguineus* and *Photuris pennsylvanica*, differ in habitat, manner of flying and of scintillating, as well as in the color of the light emitted. The males and the females each scintillate in their own peculiar manner.

2. Both species require two seasons to complete their metamorphosis, the larval life occupying the far greater part of this time. They hibernate under stones and in the soil as young and as nearly full-grown larvae. The uniformity of a brood may be broken by the varying rate of growth of the individuals.

3. The eggs of both species are subspherical, and at first sticky, and are laid among roots or just under the soil. In the labora-

tory they hatch in less than three weeks. They are faintly luminous.

4. The larva of *Photinus* is subcylindrical and slender, that of *Photuris* broad and flattened-fusiform. Both are carnivorous and nocturnal. They feed also after the second hibernation and transform into pupae in May or June.

5. The pupae retain the two larval lights on the eighth abdominal segment, and in addition, the pupa of *Photuris* has a diffuse glow, especially in the head and thorax. The pupal period probably does not exceed twenty days.

6. Both sexes of *Photuris* are active flyers and runners. The female has a voracious appetite. Only the male of *Photinus* is an active flyer.

7. The photogenic organs of all Lampyridae thus far studied are formed on the same general plan. Each organ consists of an upper or reflector layer, and a lower or photogenic layer of cells. Tracheae and nerves penetrate both layers but are more highly developed in the lower. The cuticle which overlies the photogenic organs is usually translucent.

8. There are in general two views on the derivation of the photogenic organs in Lampyridae, the one holds that they are derived from the hypodermis, and the other that they come from the fat-body. The latter view is the correct one, as Vogel ('13), and I have shown.

9. The development of the adult photogenic organ was studied in *Photinus consanguineus*. Here it is first seen in larvae near pupation. It is derived directly from the fat-body by the expulsion of its cells, which migrate to the ventral hypodermis. This cell-expulsion is effected or aided by the inflection of an investment which partly envelopes the fat-spheres here as also especially in the thoracic region. Numerous haemocytes apply themselves to these investments. No cell division was noticed during this process.

10. The cells thus liberated from the fat-spheres to form the photogenic organ, accumulate as an indefinite mass on the hypodermis and there proliferate. Two layers are gradually formed. During this process, the large albuminous granules common to

the fat-body, disappear in the forming light-organ. The reflector layer forms a sort of pavement epithelium of smaller, more clearly defined cells, while the photogenic layer is made up of larger, more rounded cells with more obscure limits. Small urate crystals occur in the upper, while minute photogenic granules darken the lower layer.

11. Tracheae and nerves enter both layers. In the photogenic layer, tracheal end-cells are developed. These end-cells are penetrated by nerves. The tracheal end-cells have slender capillary processes which seem to be intercellular and frequently anastomose.

12. The photogenic organs of the larva develop as evident structures, in late embryos. They are derived from neighboring fat cells, by migration and proliferation. As in the adult, the organ differentiates into two layers but the development is more obscure. The larval light-organ does not function in the adult. It appears to be a more primitive and less highly modified structure than the adult organ, and to be independent of the latter. In some species, as *Ellychnia* (infrequently), and *Lampyris*, it may grow in the adult.

13. The physiology of the photogenic organs has been much studied. The general belief is that photogeny is a process of oxidation of the photogenic granules, the oxygen being furnished by the tracheal end-cells. The process is dependent upon the nervous system, but photogeny may also be effected by local stimulation. Dried organs may be made to shine upon contact with water.

14. The photogenic granules are not fat-like but are probably an albuminoid which is insoluble in water and in fat-solvents. They have been regarded by some as luminous symbiotic bacteria.

15. The function of the larval photogenic organs is uncertain, but in the adult their purpose is to bring the two sexes together.

16. The germ-band of the embryo is of the immersed type, i.e., with yolk interposed between the amnion and the serosa. The amniotic cavity is formed before gastrulation occurs.

17. The embryo is at first almond-shaped, later the cephalic lobes appear and it becomes more and more arcuate. Finally the embryonic envelopes are ruptured, and the embryo reverses its position, assuming a coiled one.

18. The labrum is at first bilobed. The pleuropodia or first abdominal appendages are hypodermal structures, at first leg-like, but later glandular and mushroom-shaped. They finally disappear in old embryos by sinking into the body. Their function is uncertain.

19. The nervous system develops early. The neuroblasts are not very regularly arranged. An outer and an inner neurilemma is developed.

20. The stomodaeum and proctodaeum are deep, thick-walled invaginations. The midgut ultimately envelopes almost all the yolk material.

21. The primitive coelomic sacs are conspicuous, at first slit-like they later become rounded. They are shut off in each segment.

22. The pericardial cells appear to be mesodermal. They are of large size and differentiated even before the fat cells.

23. A suboesophageal body is present.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

- 1 *Photinus consanguineus* larva, first instar.
- 2 *Photinus consanguineus* larva, full-grown.
- 3 *Photinus consanguineus* pupa, lateral view.
- 4 *Photinus consanguineus* adult, male.
- 5 *Photinus consanguineus* male and female, ventral view of abdomen. The shaded area represents the photogenic organ. *LO*, remnant of larval photogenic organ.
- 6 *Ellychnia corrusea*, egg showing oil-globules at surface.

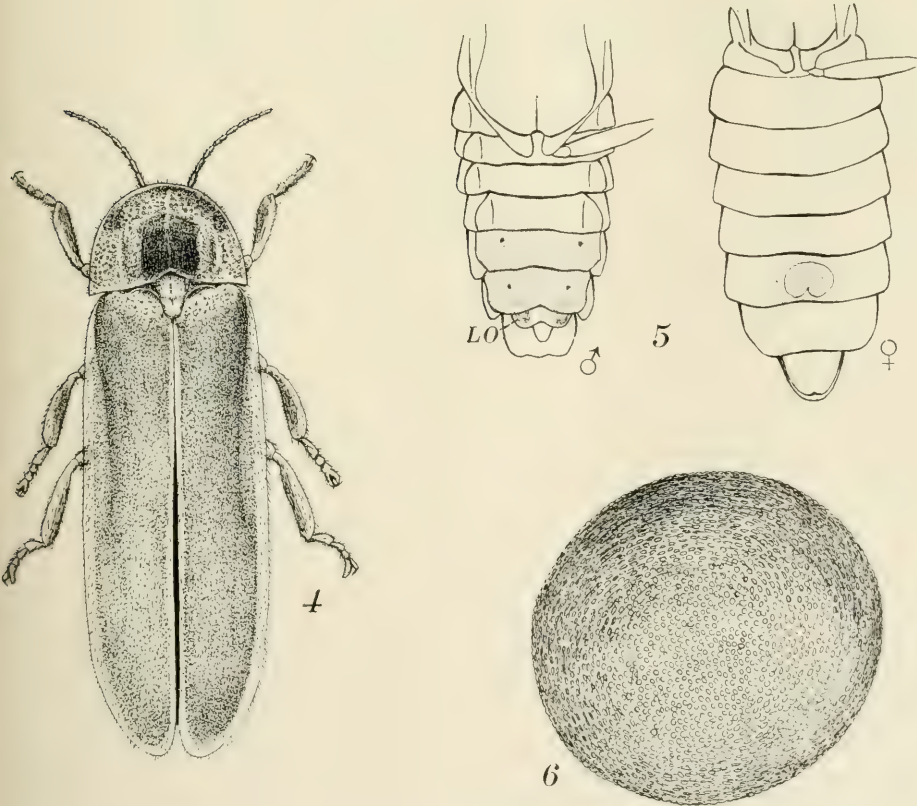
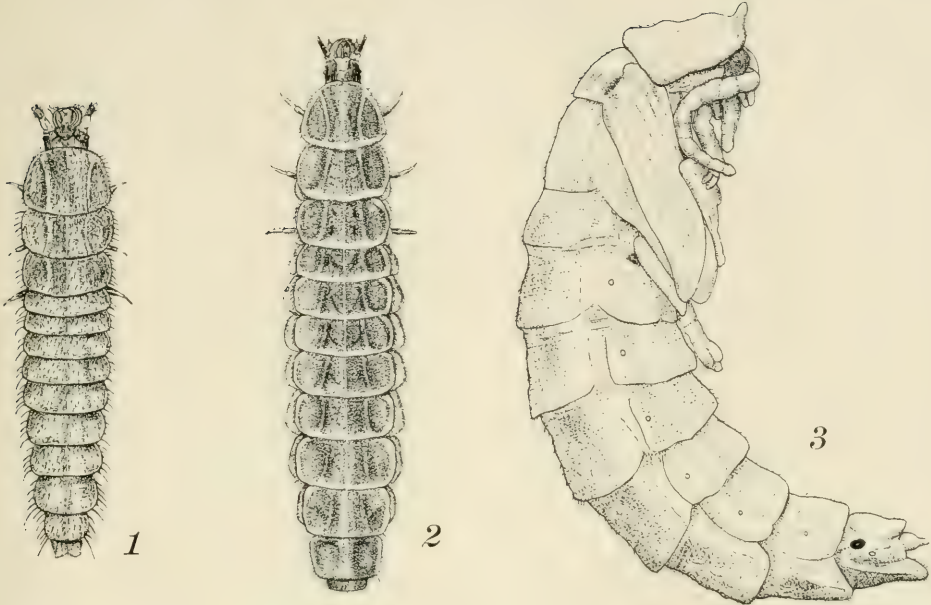
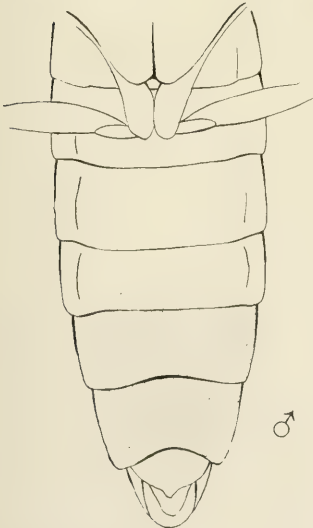
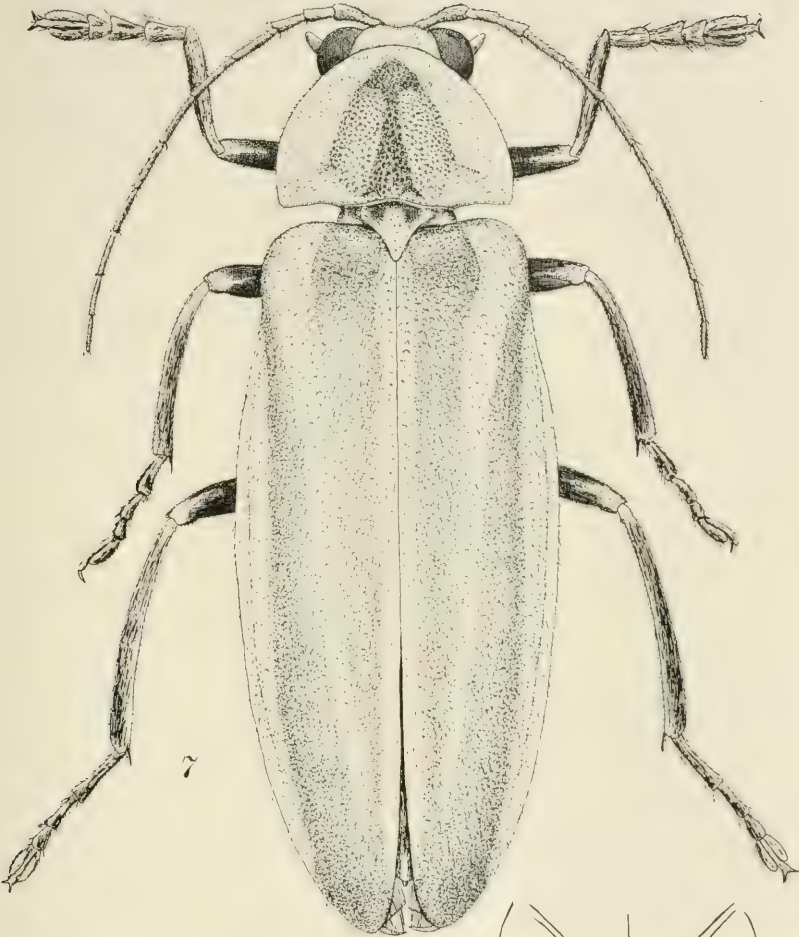


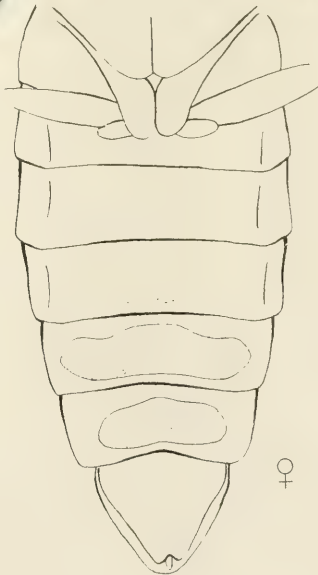
PLATE 2

EXPLANATION OF FIGURES

- 7 *Photuris pennsylvanica*, female.
- 8 *Photuris pennsylvanica*, male and female, ventral view of abdomen. The shaded area represents the photogenic organ.



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PLATE 3

EXPLANATION OF FIGURES

- 9 *Photuris pennsylvanica* larva, first instar.
- 10 *Photuris pennsylvanica* larva, full-grown.
- 11 *Photuris pennsylvanica* pupa, ventral view.
- 12 *Photuris pennsylvanica* larva, full-grown, ventral view. *P*, photogenic organ; *S*, spiracle.
- 13 Cross section of photogenic organ of full-grown larva of *Photinus consanguineus*. *B*, blood cell; *C*, cuticula; *H*, hypodermis; *M*, muscle; *N*, nerve; *P*, luminous layer; *T*, trachea; *U*, urate or reflector layer.
- 14 Sagittal section of photogenic organ of embryo *Photuris pennsylvanica*. *A*, fat cell; *H*, hypodermis; *M*, muscle; *P*, luminous layer; *T*, trachea; *U*, urate or reflector layer.
- 15 Sagittal section of photogenic organ of embryo *Photuris pennsylvanica*, an earlier stage of development than in figure 14. *A*, fat cell; *H*, hypodermis; *M*, muscle; *O*, light-organ; *T*, trachea.

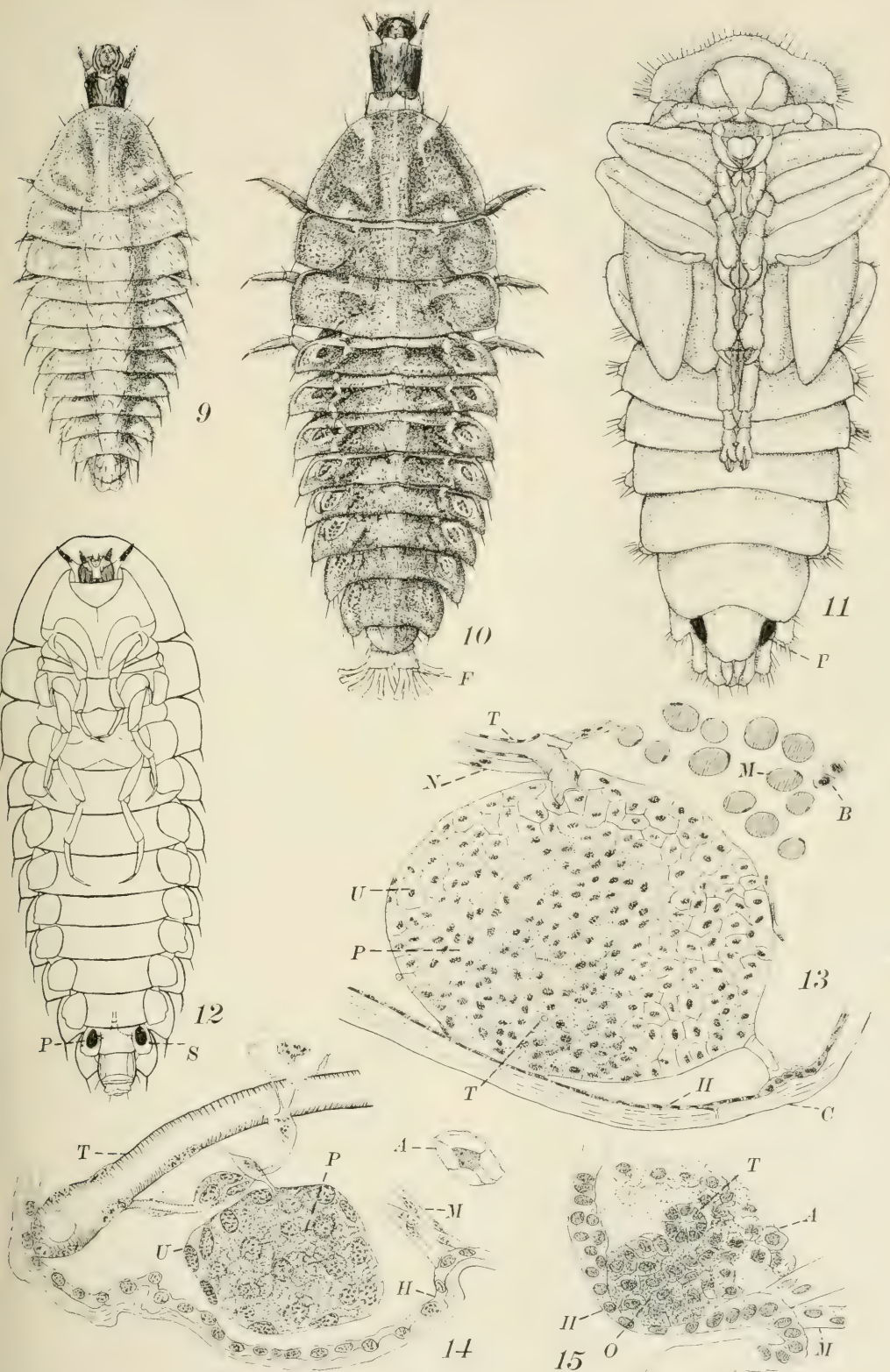


PLATE 4

EXPLANATION OF FIGURES

16 Photogenic organ of full-grown larva of *Photuris pennsylvanica*, ventral view. *N*, nerve; *P*, luminous layer; *U*, urate layer.

17 Section through a fat sphere of pupating larva of *Photinus consanguineus*. *FB*, fat haemocyte; *I*, cap or investment; *K*, nucleus of fat cell; *R*, rupture; *SB*, segmental haemocyte.

18 Section through fat sphere at a later stage than preceding; the investment *I*, is thicker and more constricted and the fat haemocytes *FB*, far more numerous.

19 Section through remnants of fat spheres and investments; lettering as in figure 17.

20 Fat and blood cells in *Photinus consanguineus*, one day out of the egg. *A*, fat cell; *B*, blood cell.

21 Fat cells in *Photinus consanguineus*, sixteen days out of the egg.

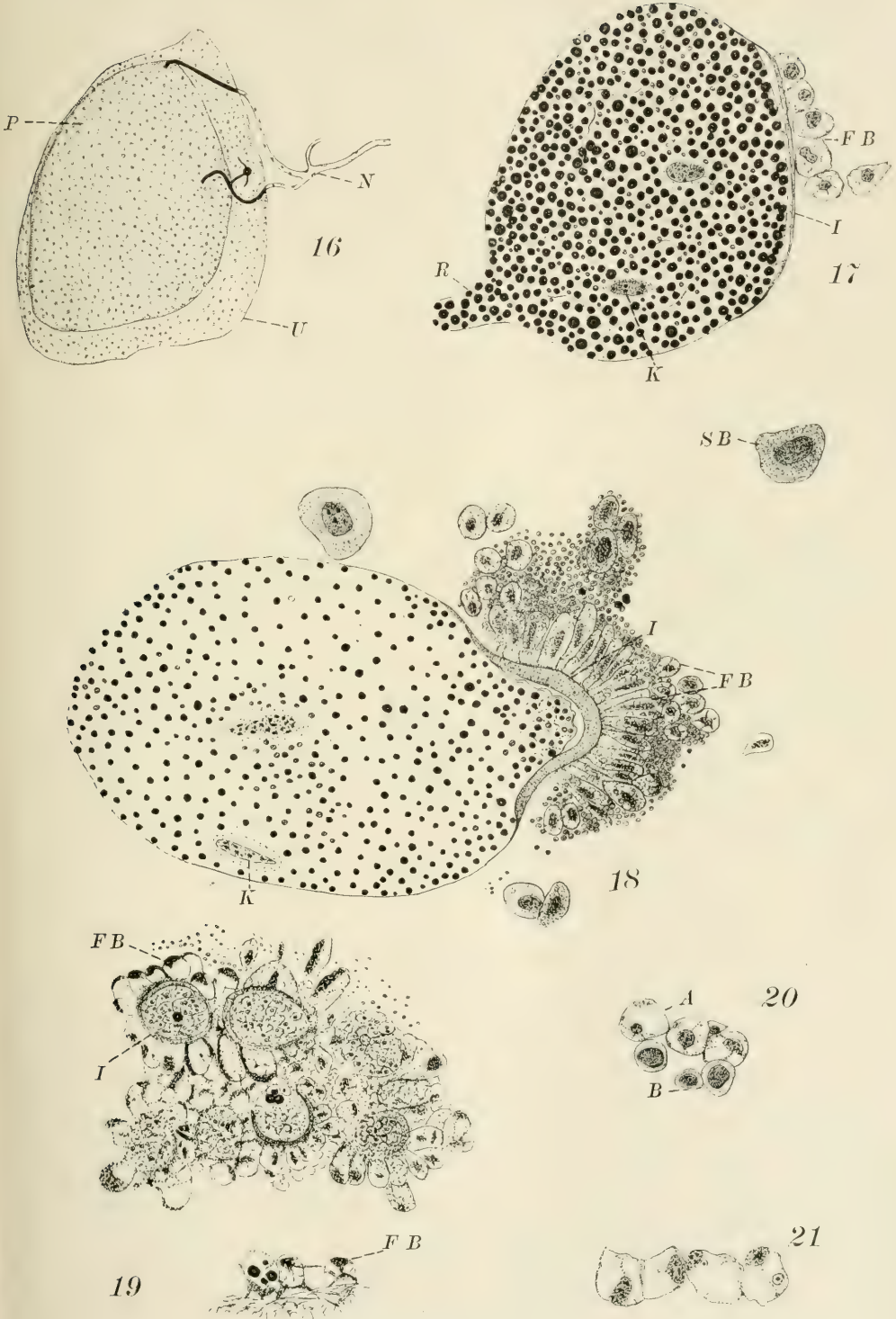


PLATE 5

EXPLANATION OF FIGURES

22 Tracheal capillary penetrating the larval photogenic organ of *Photinus consanguineus*. *c*, capillary; *T*, trachea.

23 Tracheal end-cell penetrating the photogenic layer of the adult *Photinus consanguineus*; partly from Lund. *c*, capillary; *EC*, end-cell; *T*, trachea.

24 Earliest stage observed in the formation of the adult photogenic organ of *Photinus consanguineus*; sagittal section. *A*, photogenic fat sphere, *C*, cuticula; *FB*, fat haemocytes; *H*, hypodermis; *I*, investment; *P*, photogenic cells issuing from fat-sphere; *SB*, segmental blood cells; *T*, trachea.

25 Second stage in the development of the adult photogenic organ of *Photinus consanguineus*, lettering as in figure 24; cross section; here a number of the photogenic cells have migrated to the hypodermis.

26 Cross section through the region of the photogenic organ of an early pupa of *Photinus consanguineus*, at a later stage of development than figured in 25. *A*, fat-sphere; *C*, cuticula; *D*, dorsal vessel; *H*, hypodermis; *I*, intestine; *K*, Malpighian Tubule; *M*, muscle; *N*, nervous system; *O*, oenocyte; *P*, forming photogenic organ; *T*, trachea; *V*, vas deferens.

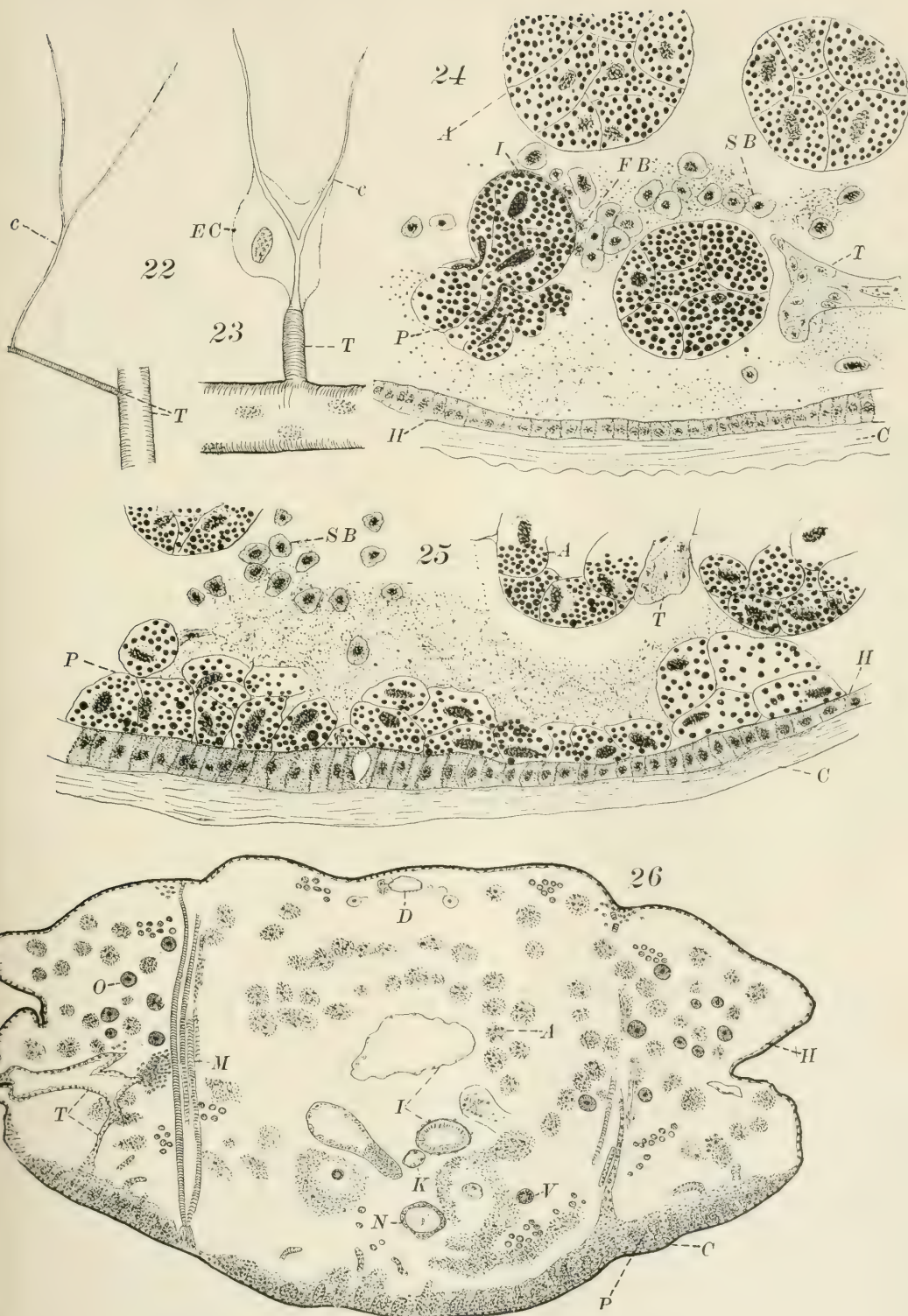


PLATE 6

EXPLANATION OF FIGURES

27 Third stage in the development of the adult photogenic organ of *Photinus consanguineus*; cross section. *A*, photogenic fat-spheres; *C*, cuticula; *H*, hypodermis; *M*, muscle; *P*, luminous layer; *T*, trachea; *U*, urate layer.

28 Fourth stage in the development of the adult photogenic organ of *Photinus consanguineus*; cross section. *A*, fat tissue; *C*, cuticula; *E*, thickened tracheal epithelium of photogenic layer, where the end-cells are formed; *FB*, fat haemocytes; *H*, hypodermis; *P*, luminous layer; *T*, tracheal epithelium; *U*, urate layer.

29 Fully developed adult photogenic organ of *Photinus consanguineus*, in adult insect; cross section. *C*, cuticula, *c*, capillaries of tracheal end-cells; *H*, hypodermis; *P*, luminous layer; *T*, trachea; *U*, urate layer.

30 Disintegrating photogenic organ of adult *Ellychnia corrusea*. *A*, fat-sphere; *C*, cuticula; *H*, hypodermis; *M*, muscle; *P*, luminous layer; *T*, trachea; *U*, urate layer.

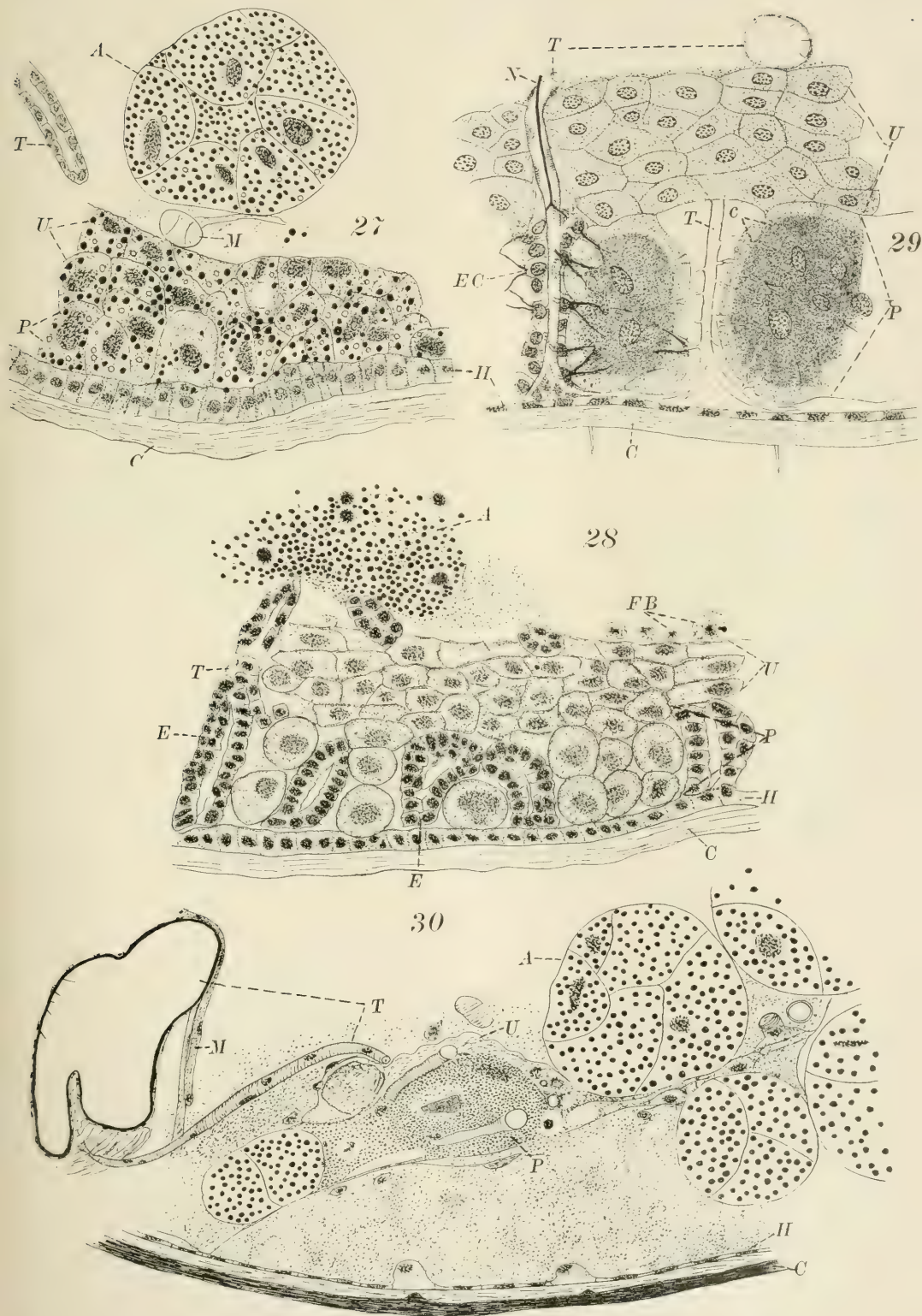


PLATE 7

EXPLANATION OF FIGURES

Embryology of *Photuris pennsylvanica*

- 31 Egg; yolk bodies represented by polygonal areas.
- 32 Section through egg; chorion not represented. *B*, blastoderm cells; *V*, vitellophags. The yolk pervades the entire egg.
- 33 Egg; divested of chorion; cross section. *A*, amnion; *B*, serosa; *G*, germ-band; *Y*, yolk.
- 34 Embryo of the same age as in figure 33; dorsal view; the broader end is the anterior one; the diamond-shaped area represents the place of union of the amnion and serosa.
- 35 Slightly later embryo than figure 34. *P*, cephalic lobe.
- 36 Lateral view of figure 35. *A*, amnion; *P*, cephalic lobes; *Y*, yolk.
- 37 Slightly later embryo than figure 35. *A*, amnion; *P*, cephalic lobes; *Y*, yolk.
- 38 Head of embryo in early segmentation; ventral view. *A*, amnion; *F*, antenna; *L*, lobe of labrum; *P*, cephalic lobe.
- 39 Embryo in later metamerization than in figure 38. *A*, amnion; *F*, antennæ; *IA*, pleuropod or first abdominal appendage; *L*, lobe of labrum; *LG*, legs; *LI*, labium; *M*, mandibles; *MX*, maxillæ; *P*, cephalic lobes; *Y*, yolk.

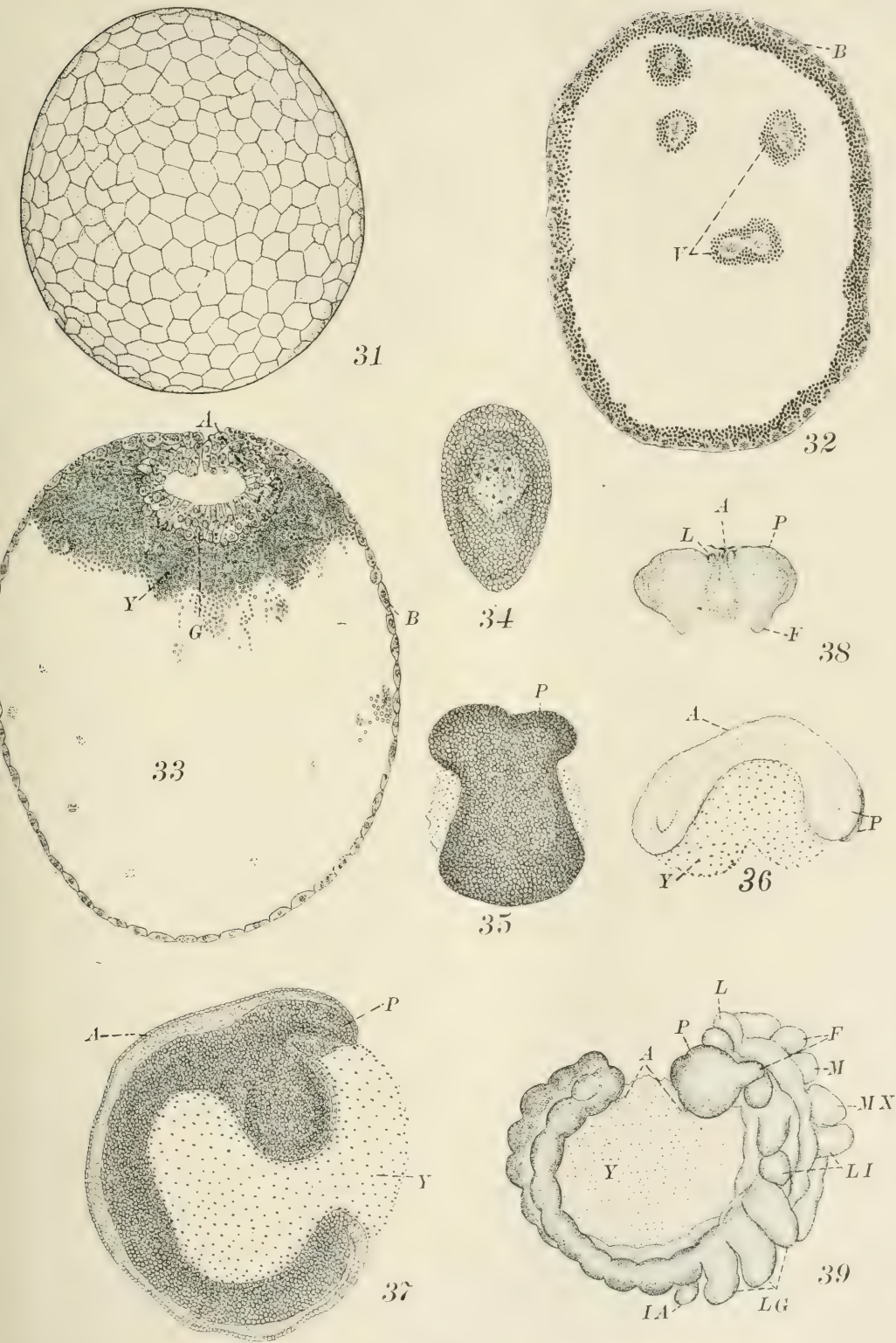


PLATE 8

EXPLANATION OF FIGURES

40 Embryo of *Photuris pennsylvanica*, straightened out; ventral view, about same age as figure 39. *A*, amnion; *F*, antenna; *IA*, pleuropod; *L*, labrum; *LG*, legs; *LI*, labium; *M*, mandible; *MX*, maxilla; *P*, cephalic lobe; *U*, mouth.

41 Embryo of *Photuris pennsylvanica*, more advanced than figure 40; lateral view. Lettering as in figure 40; *Y*, yolk.

42 An early vitellogophag.

43 Embryo of *Photinus consanguineus*; sublateral view. The embryo is commencing to curl up, beginning at the cephalic end; lettering as in preceding figures.

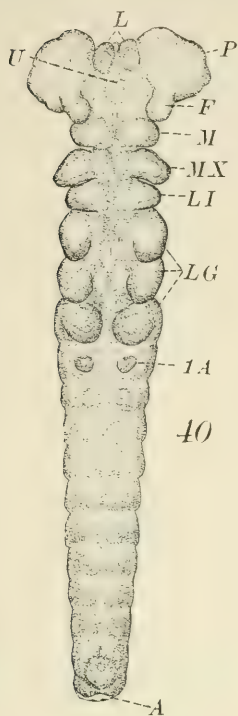
44 Same embryo as in figure 43, subventral view.

45 Embryo of *Photuris pennsylvanica*, nearly ready to hatch; lateral view. *IA*; pleuropod; *H*, head.

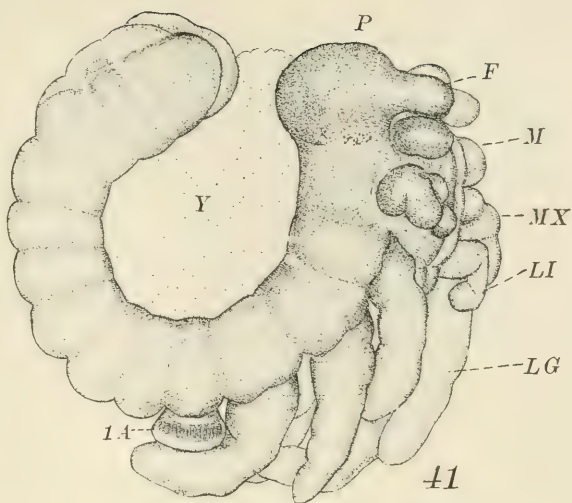
46 Cross section of Embryo of *Photuris pennsylvanica*, about the age of figure 37; head regions; *A*, amnion; *E*, ectoderm; *M*, mesoderm; *O*, amniotic cavity; *Y*, yolk.

47 Cross section of *Photuris* embryo of same age as figure 40; posterior end. *G*, neural groove.

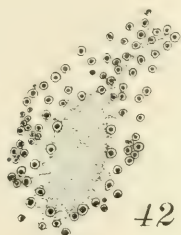
48 Cross section of *Photuris* embryo of same age as figure 47; thoracic region. *A*, amnion; *IC*, primitive coelomic sac; *E*, ectoderm; *G*, neural groove; *M*, mesoderm; *Y*, yolk.



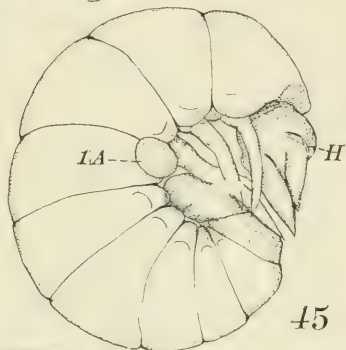
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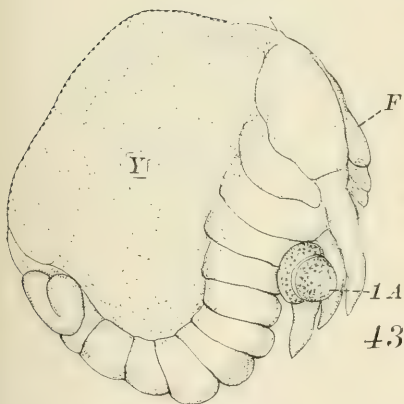
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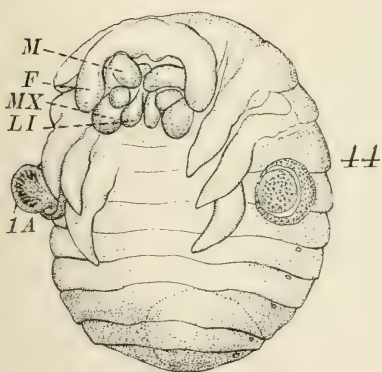
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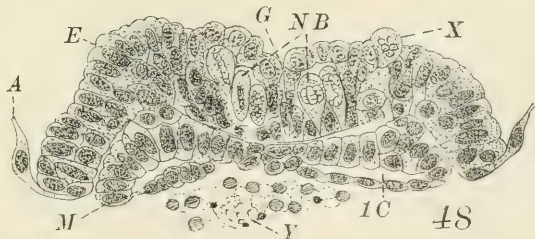
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PLATE 9

EXPLANATION OF FIGURES

Embryology of *Photuris pennsylvanica*

49 Cross section of an embryo at an age intermediate between figures 39 and 41; abdominal region. *A*, amnion; *C*, future body cavity; *IC*, primitive coelom; *Gr*, neural groove; *H*, haemocyte; *LG*, leg; *NB*, neuroblasts; *V*, vitellophag; *Y*, yolk.

50 Sagittal section through two coelomic sacs. *C*, future body cavity; *IC*, primitive coelom; *E*, ectoderm; *FB*, fat body; *H*, haemocyte; *M*, mesoderm; *OE*, oenocytes; *Y*, yolk.

51 Cross section of an embryo of about the same age as figure 41. *A*, amnion; *C*, future body cavity; *IC*, primitive coelom; *E*, ectoderm; *ENT*, entoderm of midgut; *FB*, fatbody; *G*, ganglion; *GN*, gonad; *H*, haemocyte; *NB*, neuroblasts; *O*, amniotic cavity; *OE*, oenocytes; *PC*, pericardial cells; *S*, serosa; *Y*, yolk.

52 Sagittal section of an embryo of about the same age as figure 51. *A*, amnion; *IA*, pleuropod; *BR*, brain; *E*, ectoderm; *Ent*, entoderm; *F*, punktsubstanz; *GN*, gonad; *H*, haemocytes; *IN*, inner neurilemma; *O*, amniotic cavity; *ON*, outer neurilemma; *PR*, proctodaeum; *S*, serosa; *SG*, suboesophageal body; *St*, stomodaeum; *T*, trachea; *Y*, yolk.

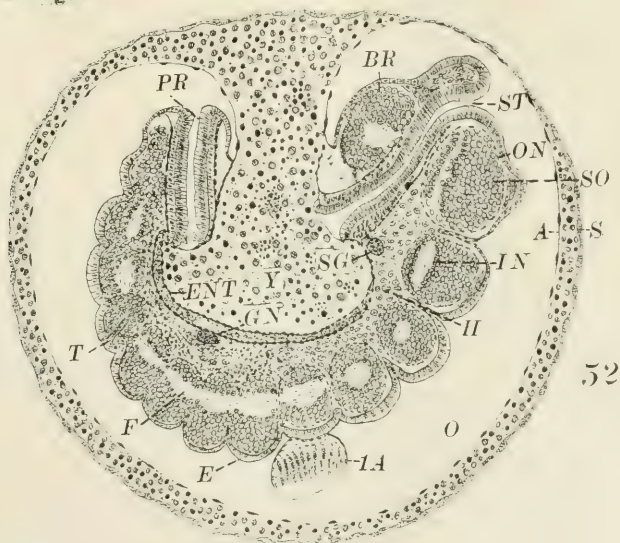
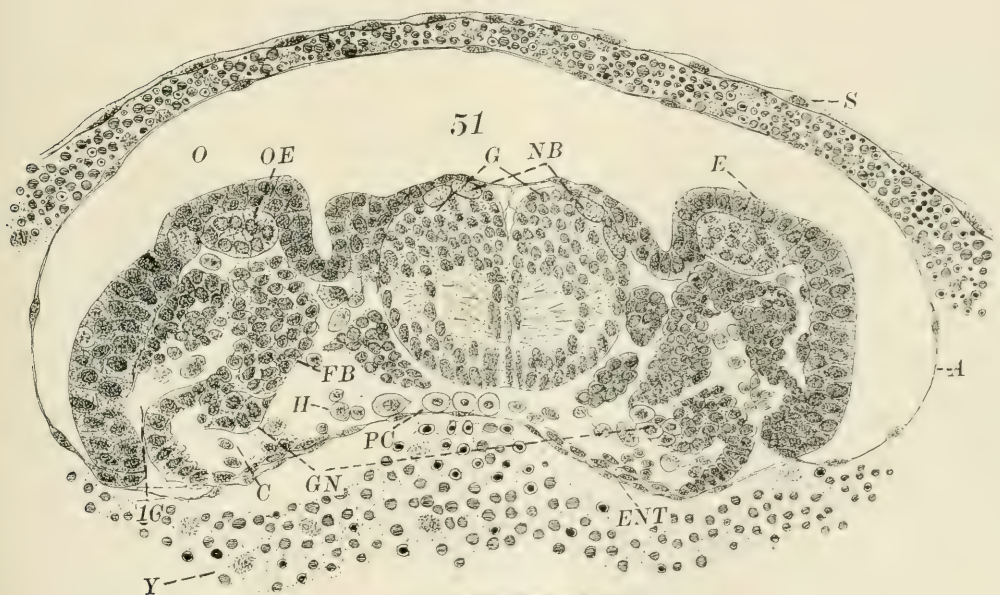
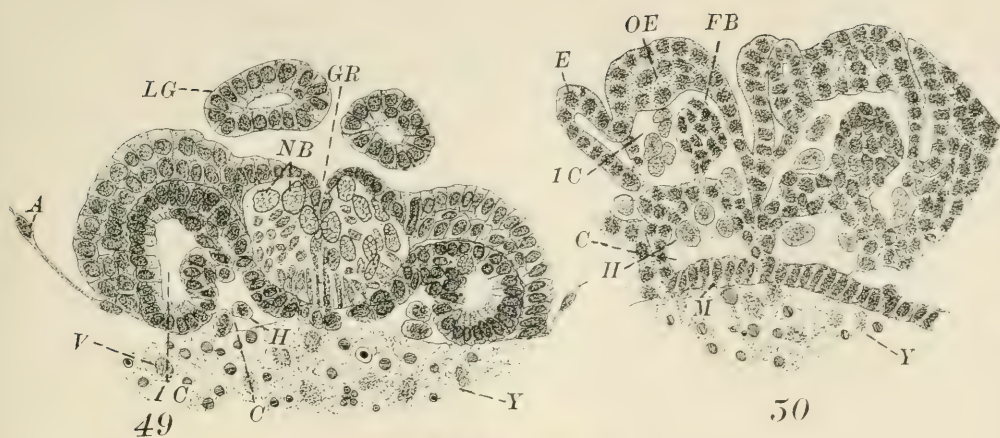


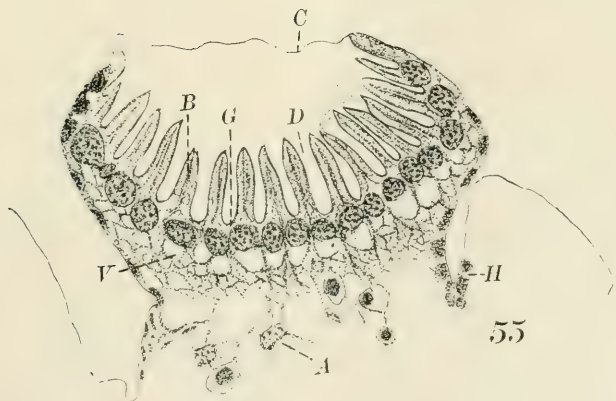
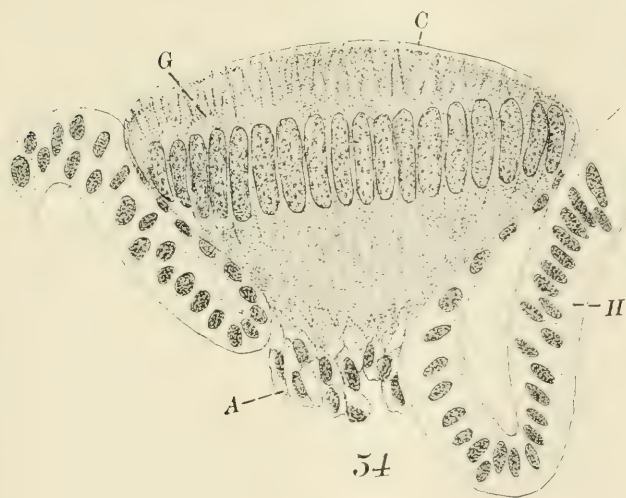
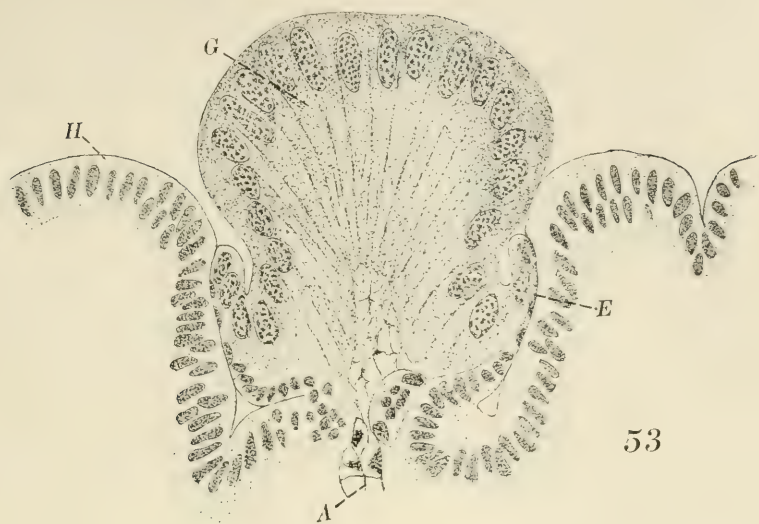
PLATE 10

EXPLANATION OF FIGURES

53 Pleuropodium of *Photinus consanguineus*; sagittal section. *A*, fat cells; *E*, basal cup; *H*, unmodified hypodermis; *G*, gland cells.

54 Pleuropodium of *Photuris pennsylvanica*; sagittal section. *A*, fat cells; *C*, cap; *H*, hypodermis; *G*, gland cells.

55 Pleuropodium of *Photuris pennsylvanica*, at a much later stage than figure 54. *A*, fat cells; *B*, boundary between two gland cells; *C*, cap; *D*, duct of a gland cell; *G*, gland cell; *H*, hypodermis; *V*, basal vacuole of gland cell.



THE DEVELOPMENT OF THE HYPOPHYSIS IN REPTILES

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SIXTY-EIGHT FIGURES

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INTRODUCTION

The hypophysis, like all other internal secreting organs, is receiving considerable attention from investigators in the fields of physiology and comparative anatomy. The importance of these organs to the animal economy can be more fully comprehended when their histological and morphological structure and development are more clearly understood. This paper is an attempt to describe the development in certain species of the various classes of reptiles and to answer some of the questions concerning such development and structure which still remain undecided. Woerdemann ('14) believes that a comparison and

homology of the hypophysis of the various vertebrates, based on the histology alone, cannot be complete. The same is true, possibly to a lesser degree, for the morphology alone. A thorough understanding of the correlated development of both phases is necessary before any homology can be satisfactory.

The material used for this study embraces the four common orders of reptiles: embryos and adults of the turtles, *Chrysemys marginata*, embryos of *Chelydra*, *Aromochelys odorata*, *Aspionectes spinifer* and *Trionyx*, and adults of *Chrysemys picta* and *Pseudemys elegans*; embryos and adults of *Alligator mississippiensis*; embryos of the lizards, *Hemidactylus* and *Lacerta agilis*, *viridis* and *muralis*, and adults of *Hemidactylus* and *Sceloporus undulatus*; while the snakes are represented by embryos of *Eutaenia sirtalis* and *Tropidonotus natrix* and adults of *Heterodon platyrhinos* and *Eutaenia sirtalis*. Observations on the various species employed are indicated in the text and in the description of the figures.

I wish to express my best thanks to Dr. C. M. Jackson of the University of Minnesota for the courtesies of his laboratories during the summer of 1915 and Dr. R. E. Scammon for the loan of young snake embryos from the Embryological Collection of the Institute of Anatomy; to Prof. H. F. Nachtrieb for permission to study the collection of turtle embryos of the Department of Animal Biology of the University of Minnesota; to Dr. F. T. Lewis for the loan of lizard embryos from the Harvard Embryological collection; to Dr. A. G. Pohlman of St. Louis University for the opportunity of studying his collection of turtle embryos; to Prof. A. M. Reese of West Virginia University for the loan of alligator slides; to Dr. E. S. Ruth for kindly sending lizard material from the Philippine Islands; and to Prof. J. F. Abbott, Dr. D. E. Jackson and Dr. Robert Gesell of Washington University for lizards, turtles and alligators. I have indicated in the description of the figures the material used and the laboratory from which it was obtained.¹

¹ The material used in the descriptions is designated as follows: H. E. C., Harvard Embryological Collection; M. U. E. C., Minnesota University, Embryological Collection; R. C., Prof. A. M. Reese's Collection; W. U. C., Washington University Collection.

LITERATURE

Embryology

In 1838 Rathke described a small outpouching from the mouth in the viper, lizard and chick and stated that it later developed into the pituitary body by the closing off of the hypophyseal stalk and the formation of a closed vesicle. Later ('39) he noted in the viper, its close relation to the brain, the numerous blood vessels—particularly the veins in the older embryos—and the carotids on either side supplying vessels to the body. The adverse opinions of his contemporaries led him to doubt his earlier observations, and in further study ('61) he concluded that the outpouching from the mouth did not develop into the hypophysis.

Sasse ('86), in a dissertation on the development and theories of the function of the hypophysis, figured the hypophysis in very young *Tropidonotus* and *Lacerta* and noted the thickening of the hypophyseal outpouching.

Orr ('87), in describing a median connection between the pre-mandibular somites, 'the coelenteric zone,' observed the intimate relation between this zone, the notochord and the hypophysis.

Ostroumoff ('88) briefly stated that the hypophysis of the lizard is of entodermal origin. Hoffmann ('86) also believed that the hypophysis, in *Tropidonotus* and *Lacerta* arises from the entoderm, but realized that this question could not easily be solved. His figures show prominent anterior and posterior portions in older embryos.

In 1890 Jägerskiöld described, in some detail, the hypophysis of *Tropidonotus natrix*. In embryos with heads 4.5 mm. in length, the hypophyseal stalk is partly constricted. A median sagittal section of the hypophysis is crescent-shaped, the anterior and posterior horns extending toward the brain, though they were not in contact with it. Measurements of the various diameters of the hypophysis and hypophyseal stalk of the embryos are given.

Oppel ('90), studying the head somites, noted the hypophyseal evagination in his 'Series II' which has 55 somites. In *Anguis*

fragilis the cellular mass connecting the premandibular somites is attached, either to the tip of the hypophysis, or directly caudal to it. Oppel was convinced of the difficulty of proving whether the hypophysis arises from the ectoderm or from the entoderm.

In 1893 Gaupp's much-quoted work on the development of the hypophysis in reptiles appeared. This has been of great interest because of his description of a tri-lobed hypophyseal anlage. Wax reconstructions of the hypophyses of *Anguis fragilis* and *Lacerta agilis* show the hypophysis developing from a median Rathke's pouch. The two lateral buds present in 2.5 mm. embryos were considered separate structures. Later, the thickened epithelium anterior to the median hypophyseal anlage evaginates, forming a four-lobed structure with one anterior, two lateral and a median or posterior lobe. The four lobes open into a common antechamber, the latter three by a common lumen. In late stages the lateral lobes lie embedded in the coverings of the brain, completely separated from the remainder of the hypophysis. The isolated masses, Gaupp believed, persist in the adult.

Valenti ('95), studying *Lacerta agilis*, *Lacerta vivipara*, and *Anguis fragilis*, readily distinguished the low, clear-staining entodermal cells and the taller ectodermal ones in the hypophysis. These cells could be recognized even after the rupture of the oropharyngeal membrane. Valenti believed that the part played by the ectoderm in the formation of the hypophysis was secondary, and that the hypophysis itself was possibly the remains of an early branchial pouch and comparable to the thymus and thyreoid.

In a *Sphenodon* embryo of 'Stage L,' about 30 somites, Dendy ('99) observed an epithelial thickening which he recognized as the pituitary anlage. In stage N the notochord curves downward in front of the hypophyseal outpouching.

In an article on rudimentary cranial appendages, Melchers ('99) considered the development of the hypophysis in *Platydictylus facetanus* as passing through three stages; in the first the orohypophysis, or Rathke's pouch, approaches the brain; in the second the orohypophysis loses connection with the mouth; and in the last stage various bud-like processes appear.

Orrù ('00) regarded the hypophysis of *Gongylus ocelatus* of both ectodermal and entodermal origin. In late stages it consists of a dorsal lobe, in contact with the infundibulum, and a ventral lobe completely separated from the infundibulum.

Staderini has published his observations on reptile hypophysis in various articles. In 1900 he reported (according to Salvi '01) that the hypophysis is connected with the head somites. Later ('03), in a paper on morphogenesis of the hypophysis of *Gongylus*, he described a primitive single anlage, which, by the development of two lateral lobes, becomes tri-lobed, and finally four-lobed. The intimate relation of the two isolated lateral lobes to the brain received this author's attention ('05), when the infundibulum was described as being divided into three parts at its caudal end. The outlines of the cells of the hypophysis are very indistinct and the round nuclei closely placed.

Studying the pharyngeal region in *Lacerta agilis*, Peter ('01) reconstructed this portion, including the hypophysis. A figure of his 'Stage 10,' a 4 mm. embryo of 47 somites, shows a body which is slightly divided into anterior and caudal parts. In the brief descriptions of the characteristics of various stages the author mentioned the appearance of Rathke's pouch in a 3 mm. embryo.

Salvi's articles ('01, '02, '06) are mainly concerned with the connection between the hypophysis and the premandibular cavities in *Gongylus*, and in attempting to explain the origin of the lateral lobes of the hypophysis.

Zeleny ('01) studied early stages of the development of the hypophysis in *Chelonia* and concluded that it is undoubtedly of epiblastic origin. In 7 mm. embryos the hypophysis is a sac-like structure connected to the pharyngeal vault by means of a slender stalk.

Gemelli ('03) described the early relations of Rathke's and Seessel's pouches in *Lacerta agilis* and other lizards and also in snakes. In 4 mm. *Lacerta* embryos the ruptured pharyngeal membrane still clearly separates the two pouches. Later, as the hypophysis increases in size, Seessel's pouch degenerates. Gemelli carefully reviewed the literature and finds that it can

be divided into four chronological periods corresponding to the prevailing theory of germ layer origin.

In 1903, Rossi, working on *Seps* and *Lacerta muralis* arrived at a number of conclusions and agreed with Gemelli ('03) that the hypophysis is of ectodermal origin.

In his 'Normal Plate' of *Lacerta agilis*, Peter ('04) described the hypophyseal anlage in 2.8 mm. embryos of 19 segments. The lateral buds are found in 3.1 mm. embryos. The hypophysis is distinctly a tri-lobed body with a single stalk at 4.2 mm.; the stalk has disappeared at 8 mm. Tubules appear soon after. No lumen is present at 10 mm.

Gisi ('07) gave an excellent description of the development and adult anatomy of the hypophysis in *Sphenodon* (Hatteria) *punctatum*. In a 15 mm. embryo there is a stalked structure with several buds, of which the anterior and caudal buds are prominent. A finger-like process is located below the caudal one. In a 19.8 mm. embryo the stalk has degenerated and the lateral buds have become compressed sagittally. In a 28.4 mm. embryo the hypophysis has grown into a tubular gland and the adult condition is present when the head is 12 mm. long.

Reese ('08), describing the development of the American alligator, mentioned a pituitary anlage in his 'stage 13' which, judging from his figure, is an embryo of about 6 mm. The development of the hypophysis received his consideration in 1910, although no anlage is described earlier than in a 7 mm. embryo. A sagittal section of a 13 mm. embryo shows a folded sac-like structure with a large outgrowth ventral to the infundibulum, a small one anterior to the first, and a third extending from the proximal end of the stalk. In later embryos Reese figured another outgrowth lying anterior to the hypophyseal sac but not connected with it. In 13 cm. alligators solid cords, composed of densely-staining cells resembling lymphocytes, are present. No lateral buds were found.

Recently Bruni (14) published a paper on the hypophysis in amniota. He studied and reconstructed the hypophysis of *Gonygylus ocellata* in various stages of development. A single hypophyseal anlage is described in 3.5 mm. embryos. Caudal to this

is Seessel's pouch, and between the two a third and smaller pouch, similar to one described by Valenti in *Lacerta*. The latter disappears, but it could not be ascertained whether it is included in the hypophyseal anlage. Rathke's pouch soon differentiates into a median and two lateral lobes. In 5 mm. embryos the anterior lobe is formed by the evagination of the epithelium, cranial to Rathke's pouch. The stalk is solid and numerous buds have appeared on the body in 10 mm. embryos. Except in the median lobe and in an anterior prolongation of the median lobe, the lumen disappears, and the lateral lobes become enclosed in the cerebral coverings, losing their connection with the hypophysis. Bruni has given some interesting figures illustrating the change in position of the hypophyseal stalk. The stalk first extends forward, then caudalward, and in older embryos, again cranialward.

Another recent article on the development of the hypophysis is by Woerdemann ('14), who believed that there is a single hypophyseal anlage, and accepted this as the view of recent investigators. From this single thickened epithelium, Rathke's pouch develops. Two lateral buds appear and still later an anterior bud. The latter is a primary portion of the hypophysis, though it appears late. All these parts are present in a 3 mm. *Chrysemys picta*. Woerdemann considers the lateral buds independent of Rathke's pouch (Gaupp's 'Mittelknospe') since the latter opens by a somewhat constricted lumen into a main cavity into which the lateral buds open separately. This cavity ('Mittelraum') is a part of the anterior end of the hypophysis and the anterior lobe opens into its cranial end. The hypophyseal stalk is attached to the ventral wall.

In a 5 mm. embryo, Rathke's pouch is closely applied to the infundibulum and is connected by a narrow neck to the anterior end of the hypophysis. The lateral lobes grow dorsalward, under or into the cranial membranes, and become divided into cell groups. The anterior lobe develops cell columns very early. The hypophyseal stalk disappears and from the 'Mittelraum' a solid bud develops, to which the lateral buds are attached in 9 mm. embryos.

To summarize: Gaupp has described a tri-lobed origin of the hypophysis. The majority of other investigators agree that there is a single anlage—Rathke's pouch—from which the hypophysis develops. Woerdemann finds an early thickened epithelial anlage from which Rathke's pouch grows caudalward and from the anterior end of which the lateral buds and an anterior lobe develop. The majority of investigators also agree that the hypophysis is of ectodermal origin, though Valenti described it as entodermal. Orrù believed the origin both ectodermal and entodermal, while Hoffman appreciated the difficulty of determining from which layer it arises. That the lateral lobes lose connection and become applied to the brain wall is generally conceded for *Gongylus*.

Although the terms applied to the various parts of the hypophysis during its development have been influenced somewhat by Gaupp's ('93) paper, a table of these terms may help to clear any misunderstanding.

Anatomy and histology

Müller ('71) described hypophyseal tubules composed of irregular cells surrounded by numerous capillaries in late turtle embryos.

Stieda ('75) found a small dorsal portion closely united to the lobus infundibuli, and a large ventral lobe, both composed of solid cell cords formed of polyhedral cells with a distinct granular nucleus. The dorsal portion is more vascular and contains pigment cells in turtles.

Rabl-Rückhard ('78) in his paper on the central nervous system stated that the hypophysis of alligators is egg-shaped and contains no cavity.

In 1890 Hoffmann referred to the hypophysis in turtles as egg-shaped and closely united with the lobus infundibuli. Stieda's terminology is employed by Hoffmann.

No direct reference is made by Saint-Remy ('92) to the hypophysis of reptiles, though he stated that he had examined lizard and turtle material. He believed that the two kinds of

TABLE 1

AUTHOR	FORM	PARTS OF HYPOPHYSIS		
Gaupp '93.....	<i>Lacerta agilis</i> <i>Anguis fragilis</i>	Vordere Knospe	Seiten Knospen	Mittel oder terminal Knospe.
Valenti, '95....	<i>Lacerta agilis</i> <i>Anguis fragilis</i>	Inferior ex- tremity	Lateral buds	Upper extrem- ity.
Melchers, '99...	Gecko	Vordere Knospe	Nebentaschen	Rathke's Tasche (Zunge).
Orrù, '00.....	<i>Gongylus</i>	Ventral lobe		Dorsal lobe.
Gemelli, '03....	<i>Lacerta agilis</i>		Lateral buds	Rathke's pouch.
Staderini, '03, '05	<i>Gongylus</i> <i>ocellatus</i>	Anterior lobe	Lateral lobes	Medial lobe.
Woerdemann, '14	<i>Chrysemys</i> <i>picta</i> <i>Croc-</i> <i>dilus</i>	Vorraum und Mittelraum	Lateral Knospen	Rathke's Tasche.
Bruni, '14.....	<i>Gongylus</i> <i>ocellatus</i>	Lobo (anteri- ore) rostrale	Lobi laterali	Lobo medio.

cells present in the hypophysis could be explained by glandular activity.

In his paper on the hypophysis and infundibulum of vertebrates, Haller ('96) found, in *Lacerta lutaria*, a small caudal part and a cranial layer, both composed of tubules interwoven with a vascular connective tissue. The tubules contain very fine lumina. The cylindrical cells, light-staining and granular, have a distally placed nucleus. The gland tubules of the caudal part project upward and forward, as in *Emys*, and those of the cranial part upward and backward. Some tubules open dorsally into a space, flattened dorso-ventrally, from the anterior end of which is the opening leading to the subdural space which Haller has described in all vertebrates. The hypophyseal cleft is lined with gland cells. *Lacerta* differs from other reptiles in having no anterior lobe, although the hypophysis may be long, as in *Chelonia*.

Edinger, ('99) briefly described the infundibulum and saccus vasculosus of reptiles, and noted the close connection of the hypophysis with the latter.

In 1904 Sterzi's paper on the structure of the hypophysis appeared. He carefully described the histology of the hypophysis in several Saurians and Chelonians and in one alligator. In all, Sterzi found a small median as well as an inferior portion, and a nervous, superior portion. The median lobe in Saurians forms a stratum around the nervous lobe. No lumen could be found in this lobe. The cells are arranged in masses. Some of the cells are large and elliptical with spherical nuclei, poor in chromatin but containing a large nucleolus. The cytoplasm stains lightly. Other cells are small, with dark-staining nuclei, and appear compressed between the large cells. The inferior lobe is large and has small cells which contain numerous granules with an affinity for stain. Here the cells are arranged in columns between which are numerous blood vessels. In *Emys lutaria* and *Testudo graeca* the inferior part has two small lateral lobes at the caudal end and a small anterior prolongation lying against the floor of the brain. The cells are arranged in cell cords smaller than those in the lizards. The cytoplasm is full of chromophilic granules, although numerous cells are found which contain few or no granules. The nuclei are spherical and contain nucleoli. The capillaries are surrounded by connective tissue. The median portion is a thin stratum interposed between the inferior lobe of the brain and the inferior lobes of the hypophysis and separated from the former by a distinct connective tissue stratum. The cells are of two kinds, are arranged in lamina, and are chromophobic. In one specimen of alligator, Sterzi found the median lobe small and the anterior diverticulum lacking. Sterzi has described median and inferior lobes in reptiles as in other vertebrates, the first being chromophobic and small, the latter chromophilic and large. The cavity described by Haller ('96) was not observed by Sterzi, nor was any evidence found of an opening to the subdural space.

Gentes ('07) stated that the juxta-neural and distal parts of the hypophysis of reptiles are partially separated by a fibrous lamina, and in Chelonia by a cleft, at the periphery of which the two parts fuse.

Gisi ('08) described the hypophysis of *Sphenodon* as an ovoid body, more or less divided into a ventro-anterior portion of darkly-staining strands with few capillaries, and a caudo-dorsal mass composed of thinner columns and more vascular than the former. In the ventro-anterior portion some cells have large elliptical nuclei with much cytoplasm, others have little cytoplasm. The former are preponderant in the caudo-dorsal portion. A richly vascular third part, composed of few cords, and enclosed within the pia mater forms a lobus terminalis. In *Testudo graeca* and in *Emys* the axis of the hypophysis is horizontal while in *Hatteria* it is oblique. In *Lacerta*, Gisi found no lobus terminalis, and in *Chamaeleon* the orohypophysis is smaller than the nervous portion.

In discussing the histologic appearance of the hypophysis of the turtle, snake and alligator, Tilney ('11) describes a juxta-neural and a distal epithelial portion in reptiles as in other vertebrates. The dorsal juxta-neural part of turtles contains a cavity and is formed of basophiles. The larger distal part has an accessory tongue-like process extending forward from the lower anterior angle, and is composed of acidophilic cells, most of which are deeply-staining. The hypophysis shows an apparent acinal arrangement of the cells, with masses of basophilic cells between the acini. An homogeneous substance, staining deeply with eosin, is present in some lumina. The same parts are present in *Eutaenia sirtalis*. In the distal epithelial portion the cells are acidophilic and faintly staining, with granular nuclei and distinct cell membranes. No blood vessels, connective tissue, or colloid material are found in any part of the gland. In alligator an accessory process, extending forward toward the chiasma, and acini containing colloid, were noted.

Stendell ('13) described the 'Zwischenlappen' of *Sauropsida* as very small and closely attached to the infundibulum. The cells are light staining and polygonal. In turtles he found numerous cyst-like tubules in the 'Hauptlappen,' whose lumina were filled with a secretion. Such secretion was not observed in young individuals.

Herring ('13) briefly described the histological features in adult *Testudo graeca* and *Lacerta viridis*. He recognized an epithelial lobe, separated caudally from a pars intermedia. The epithelial lobe has an acinous, or cord like arrangement of cuboidal or columnar cells, most of which are clear, others deeply granular. The latter frequently form solid columns, while the clear cells surround a lumen containing a stainable colloid. This portion is very vascular. The pars intermedia is a solid band of clear epithelial cells lying at the base of the brain and covering the anterior end of the glandular part. The cells of the pars intermedia are arranged in columns, or occasionally, as acini containing colloid, and form several layers at the base of the brain. Caudally, they form a single layer of columnar cells. Herring believes the colloid is stored secretion.

Sauerbeck ('05) in describing the hypophysis of a *Sphenodon* embryo with a malformed brain, briefly stated that the hypophysis was made up of scattered epithelial cells, arranged in cords extending from the abnormally high infundibulum to the pharyngeal roof. The malformed condition of the brain was probably responsible for the elongated hypophysis.

Viguier ('11) studied the histological appearance of the hypophysis of the lizard, *Uromastix acanthinurus*, after thyroidectomy. Normally, the hypophysis is formed of epithelial cords surrounded by some connective tissue and capillaries. Clear and vacuolated and darkly-granular cells form acini with walls two or three cells deep, the central layer being flattened about a lumen. Eight or ten weeks after thyroidectomy, cells with basophilic granules, and others staining deeply with picric acid are present. Peripheral acidophilic cells surround the capillaries and may contain dark-staining granules. The nuclei are large and contain nucleoli. The capillaries are greatly dilated and congested.

The kinds of cells present, and their arrangement and relations, have been variously described. The lack of comparison of the structure and histogenesis of the hypophysis is apparent from this review of the literature. As various names have been applied to the parts of the adult reptilian hypophysis, a table

of investigators and the terms they have employed in describing the various parts of the reptilian hypophysis, together with the species studied, may be of value (p. 222).

A review of the literature shows that various questions are still unsettled. Regarding the development Rathke's pouch has very generally been thought to form the anlage of the hypophysis, but whether the notochord takes any part in its formation has not been determined. Gaupp believed the lateral buds to be separate outpouchings. Their ultimate fate has been described by Gaupp in lizards and by Gisi in *Sphenodon*. Whether in other reptiles they form a *pars terminalis*, as Gisi thought probable in *Sphenodon*, or whether the tongue-like process is formed from the anterior lobe (Stendell '14), remains in doubt. Reese stated that lateral buds are absent in alligator embryos, but Tilney observed a tongue-like process in adults. Lateral buds have not been described in snake embryos. In *Gongylus*, Bruni believes the *pars intermedia* develops from the tip of Rathke's pouch. A lumen between the *pars intermedia* and the anterior lobe or in the *pars intermedia* itself, has been observed in various reptiles (Tilney, Stendell) although its presence has been denied in others (Sterzi). Staderini described the isolated masses derived from the lateral buds in *Gongylus* as being histologically like the remainder of the hypophysis, although this cannot be confirmed by my material.

MORPHOGENESIS OF THE HYPOPHYSIS

Turtles

In transverse sections of a 2 mm. *Chelydra* embryo, there is a thickened hypophyseal plate showing no evagination. A median evagination projecting forward against the brain wall is present in a 2.6 mm. embryo.

The following description is based on a study of *Aromochelys odorata* and *Chrysemys marginata*. Wax reconstructions were made of several of the former, while the latter were studied in section.

TABLE 2

Table of terms applied to the parts of the adult hypophysis

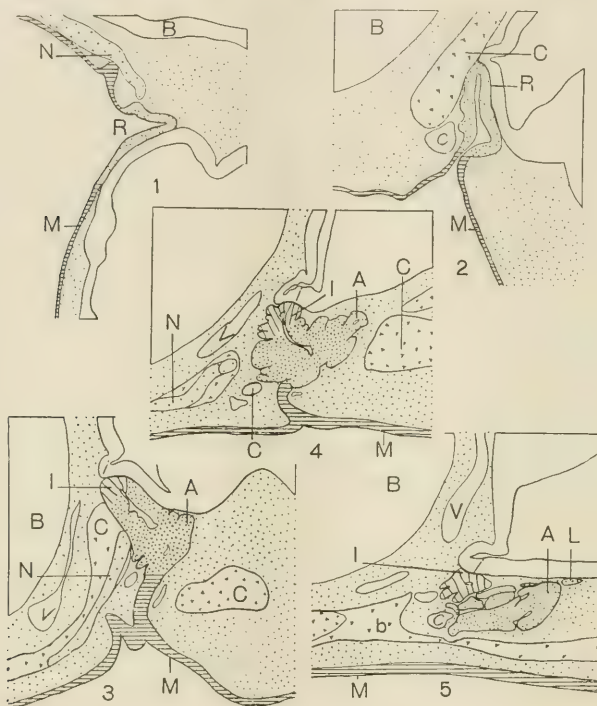
AUTHOR	FORM	PARTS OF HYPOPHYSIS		
Müller ('71) . . .	Testudo graeca	Obere Lappen	Hauptlappen	
Stieda ('75) . . .	Schildkröten	Obere Lappen	Untere Lappen	
Gaupp ('93) . . .	Lacerta agilis	Terminal	Vordere	Lateral
	Chelone	Knospe	Knospe	Knospen.
Haller ('96) . . .	Lacerta agilis	Sammelslauch	Hypophysen-Körper	
	Emys lutaria	Sammelslauch	Hypophysen-Körper	Vordere Lappe
Sterzi ('03) . . .	Alligator	Porzione media	Porzione inferiore	
	Lacerta viridis	Porzione media	Porzione inferiore	
	Testudo graeca	Porzione media	Porzione inferiore	Prolungamenta anteriore.
Gisi ('07)	Hatteria	Lobus medius Obere Teil	Lobus medius Ventral Teil	Lobus terminalis.
	Testudo graeca			
	Chameleon	Lobus medius	Lobus medius	Lobus terminalis.
	Lacerta ocell.	Lobus medius Obere Teil	Lobus medius Ventral Teil	
Tilney ('11) . . .	Cistudo caro.	Juxta-neural part	Distal epithelial part	Tongue - like part.
	Alligator	Juxta-neural part	Distal epithelial part	
	Eutaenia sirt.	Juxta-neural part	Distal epithelial part	
Stendell ('13, '14)	Hatteria	Zwischenlappen	Hauptlappen	
	Sauria	Zwischenlappen	Hauptlappen	
	Crocidilia	Zwischenlappen	Hauptlappen	
	Ophidia	Zwischenlappen	Hauptlappen	
	Chelonia	Zwischenlappen	Hauptlappen	Zungenförmigen Fortsatz.
Herring ('13) . .	Testudo graeca	Pars intermedia	Pars glandularis	
	Lacerta viridis	Pars intermedia	Pars glandularis	

In an *Aromochelys* embryo, 3.5 mm. long, there is a single, median dorsal outpouching (Rathke's pouch) from the roof of the mouth, which extends slightly forward toward the diencephalon. The lateral walls of the pouch converge toward a broad blunt apex which is about one-third as wide as the base. The cranial surface is slightly concave and the dorsal surface convex. The opening into Rathke's pouch is a wide horizontal slit.

In a 4.5 mm. embryo the anlagen of the lateral buds are well marked. These are situated on either side of the hypophyseal outpouching and are separated from the median portion by slight furrows on the cranial side (fig. 60). A careful comparison with plastic reconstructions of younger and older embryos shows that these buds are not new outgrowths from the mouth, as Gaupp believed them to be, but are constrictions from the sides of Rathke's pouch. Although the notochord in this and later stages is usually in direct contact with the caudal surface of Rathke's pouch, in this specimen there is a projection on the caudal side, near the base of the median part, to which the notochord is applied (fig. 1). Whether this projection has any connection with a constant growth from the caudal wall in older embryos (to be described later) is uncertain. Because of the constriction of the lateral buds, the base is about the same width as the apex, the lateral walls extending perpendicularly to the apex. The opening into the median hypophyseal outpouching is about half as wide as it was in the earlier (3.5 mm.) stage. Looking into this opening, as presented in the model, a deep median lumen and two shallow lateral pockets may be seen. The pockets are formed by the constriction of the lateral buds. The three parts are continuous, however, and form the common lumen of the hypophyseal anlage.

In a 5.2 mm. embryo, the median portion or Rathke's pouch is increased in length. The caudal surface is still concave and the cranial surface convex. The apex is flattened dorso-ventrally, and the lower portion, between the finger-like lateral lobes, is constricted and circular in outline. The lumina of the lateral lobes are relatively smaller than the median lumen, which is

wide at the apex, circular in the constricted portion, and again widening at the oral end where it receives the openings of the two lateral buds. The lumina of the three outpouchings appear as branches of a common hypophyseal cavity. The oral epi-



Figs. 1 to 5 Median sagittal sections of the hypophysis of turtle embryos: 1, 4.6 mm.; 2, 6.4 mm.; 3, 9.2 mm.; 4, 14.5 mm.; 5, 21 mm. Figure 3 is from *Chrysemys marginata*, others from *Aromochelys odorata*. $\times 40$ (W. U. C. 79, 77, 91, 97, 46). A, anterior lobe; I, pars intermedia; L, lateral lobes; b, basis cranii; B, brain wall; c, anastomosing branch of carotids; C, cartilage; i, infundibulum; M, epithelium of mouth and stalk of hypophysis; N, notochord; R, Rathke's pouch; v, veins.

thelium, directly anterior to these three outpouchings, is thickened and slightly evaginated, forming the anlage of the anterior bud.

In a 6.2 mm. embryo, the furrows separating Rathke's pouch and the lateral lobes are very wide. The oral epithelium rostral

to the original outpouching is distinctly evaginated and slightly constricted from the oral roof, forming the anterior bud (fig. 2). The lumina of the median and lateral lobes are continuous with the larger cavity of the anterior bud, the wall of which is thicker than that of the earlier outpouching. The oral roof is slightly raised at the region of the hypophysis, indicating the beginning of a stalk.

The lateral lobes are shorter and thicker in the 5.5 mm. *Chrysemys* than in the 5.2 mm. *Aromochelys*, and more prominent in the 6.5 mm. *Chrysemys* than in the 6.2 mm. *Aromochelys*. The anterior bud, in the 6.5 mm. *Chrysemys*, is not distinctly evaginated. These may be individual variations.

The hypophysis is well separated from the oral epithelium by a stalk in older embryos. In a 7.2 mm. embryo, a short stalk with a small lumen extends from the ventral wall, near the caudal end of the hypophysis, slightly backward and downward, to the pharyngeal vault (fig. 61). The anterior lobe is very short. The dilated lateral lobes open by means of slightly constricted necks into the caudal end of the anterior lobe. Their free ends extend anteriorly beyond Rathke's pouch. Two small solid outgrowths from the caudal wall of the median lobe are present. The dorsal one is similar in position to the projection in younger embryos, with which the notochord came in contact. The ventral bud, also solid, lies just above and cranial to the small transverse anastomosis of the carotids. The apex of Rathke's pouch is crowded between the spheno-occipital cartilage and the cranial wall. The lower lateral walls of the lateral lobes are indented by the carotids.

Marked changes have taken place in slightly larger embryos. In an 8.5 mm. embryo the stalk, containing a small lumen, is long and tube-like. One of the solid outgrowths on the caudal surface of the hypophysis is more prominent. Above this the tip of the notochord lies close against the hypophyseal wall. The wide free apex of Rathke's pouch is closely applied to the infundibulum and shows evidence of beginning glandular development. Its center is constricted, and the attached end enlarged dorso-ventrally. The anterior lobe is also enlarged. The lateral

lobes have increased in their cranio-caudal diameter, and their attachment to the anterior lobe has grown cranialward. Their free ends extend distinctly upward and almost at right angles to the median lobe.

A 9.2 mm. embryo has a longer hypophysis, the increase in length being marked in the median portion, or Rathke's pouch (fig. 3). The dorso-ventral diameter, especially that of the anterior lobe, is greater. The stalk is elongated and its lumen is just perceptible, except at its attachment to the hypophysis, where a distinct dilatation is found. Several small glandular outgrowths are present, the one near the notochordal tip having a small lumen. The basi-sphenoid cartilage presses against the caudo-ventral wall of the hypophysis. In a 9.5 mm. *Chrysemys* embryo, the lateral lobes are attached to the ventro-lateral side of the anterior lobe.

The hypophyseal stalk in a 14.5 mm. embryo is short, solid and extends caudalward and upward, showing evidence of degeneration near its attachment to the hypophysis (fig. 4). The anterior lobe has many glandular outgrowths from all its surfaces and the central lumen has almost entirely disappeared. The upper wall of the apex of Rathke's pouch has grown forward, giving the appearance of a new growth lying just below the infundibulum. A constricted neck connects this with the remainder of Rathke's pouch, which now resembles the anterior lobe in structure. The lumen of the apical enlargement is continuous with that of the central portion of the anterior lobe.

In a 17 mm. *Aromochelys* embryo, the long axis of the hypophysis extends cranio-caudally and almost in a horizontal plane (fig. 62). Many irregular, cord-like growths from the surface of the hypophysis, have made their appearance. The flattened apical enlargement of Rathke's pouch, which in previous (8 mm.) stages was somewhat constricted from the remainder, lies dorsal to the caudal end of the pouch. Cords extend laterally, anteriorly and caudally from this enlargement. The main part has a small lumen. Ventral to this portion of Rathke's pouch, and cranial to the constriction, many irregular cell cords extend caudalward. A ventrally-lying cyst-like mass contains

a small lumen, and apparently is the remains of the upper end of the hypophyseal stalk. The definitive anterior lobe is composed of the early anterior bud and the anterior end of Rathke's pouch. The anterior lobe is circular, with an irregular surface, due to the cell cords projecting out in various directions.

The lateral lobes have grown decidedly forward and dorsalward. Their distal ends are flattened, presenting a wing-like form, and extend forward against the brain floor. The proximal parts, crescentic in cross section, lie lateral to the anterior lobe, and are attached to its ventro-lateral wall (fig. 62). A lumen is no longer present in the lateral buds.

The apical enlargement of Rathke's pouch, now the pars intermedia, is widened greatly in a 20 mm. *Aromochelys* embryo (fig. 5). Many cords extend laterally under the branching infundibular outpouching. The pars intermedia is continuous ventrally with the anterior lobe.

The anterior lobe in a 28 mm. embryo has a star shaped appearance in transsection, the arms being sometimes enlarged. This and the lateral lobes lie near the brain floor (fig. 6). The proximal parts approach each other, encircling the middle region of the anterior lobe (fig. 7). The pars intermedia has also elongated. A transverse section (fig. 8) shows cord-like outgrowths and a narrow connection with the anterior lobe. Its close relation to the overlying infundibulum is also shown.

In young adult *Chrysemys marginata*, the hypophysis has elongated, measuring 0.5 mm. or more by sections. Its caudal end lies between two thin lamellae of bone, the lower one forming the roof of the mouth. An anastomosing branch joining the two cerebral carotids, lies partially imbedded in the caudal end of the hypophysis. The carotids lie close to the lateral sides, in the angle between the infundibulum and the main part of the pituitary body. A loose connective tissue surrounds the hypophysis, while laterally and in front are some of the eye muscles. The oval anterior lobe lies close against the brain floor. The distal portions of the lateral lobes are now continuous across the median line, and form a crescent-shaped lamella applied to the brain floor, and extending beyond the anterior lobe. Ven-

trally, this lamella of cells is continuous with the proximal portions which surround and fuse with the anterior lobe. Caudal to this fusion, between the hypophysis and the brain, is a layer of vascular connective tissue separating the pars intermedia from the anterior lobe. Farther back the two lobes are closely united. The pars intermedia is closely attached to the floor of the infundibulum, which is divided into several branches, partially separated by projections of the pars intermedia.

In one specimen of a young adult *Chrysemys*, a small dilated tubule, about 0.5 mm. long, originating caudal to the middle of

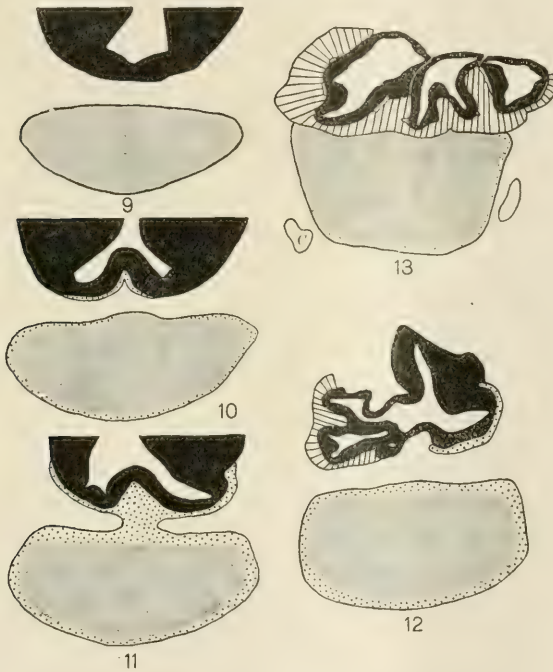


Figs. 6 to 8 Transverse sections of the hypophysis and brain floor of a 28 mm. *Aromochelys*. $\times 40$ (W. U. C. 99). Fine stipple, anterior lobe; coarse stipple, lateral lobes; black, brain and infundibulum; C, carotid artery.

the hypophysis, is imbedded in the ventral surface of the anterior lobe. Leaving the hypophysis it extends forward and downward, is continued through a small canal in the basis cranii for about half its length, and ends immediately below the floor of the cranium near the anterior end of the hypophysis. The epithelium of the oral roof is not included in the sections, but the tubule appears to end blindly in front.

The hypophysis in adult *Chrysemys elegans* and *Pseudemys picta* forms a large ovoid body, closely applied to the floor of the brain. Its length is about 3.4 mm. to 4.0 mm. and its width, 1.0 mm.

In gross dissection, only the anterior lobe is seen from the ventral side. This part ends cranially in a blunt point, caudally it is oval in outline and rectangular in cross section. Near the caudal end, on either side, lie the cerebral carotid arteries (fig. 12) joined caudally by an anastomosing branch which lies against the posterior end of the anterior lobe. Near the center, on either side, lie striated muscle masses, probably some of the eye



Figs. 9 to 13 Transverse sections of the hypophysis and brain floor of an adult turtle. $\times 20$ (W. U. C. 200). Stipple and lines as in figure 6.

muscles. The bony cranium extends upward, encircling the caudal end of the hypophysis. A definite layer of connective tissue separates the anterior end from the floor of the brain. A sagittal section shows two areas of contact between the hypophysis and the brain, a caudal one to the infundibulum, and an anterior one to the floor of the third ventricle (fig. 45). A series of drawings of a transversely sectioned hypophysis was

made to show the relations of the various parts. The first drawing shows the oval end (fig. 9) lying ventral to the floor of the brain. A second drawing of a section further caudally shows the anterior lobe almost surrounded by the proximal parts of the lateral lobes. A thin layer of the distal portions of the lateral lobes is applied to the floor of the brain (fig. 10). Figure 11, almost 2 mm. from the anterior end, shows the anterior lobe completely surrounded, with the distal portion lying against the floor of the brain. The anterior lobe of the hypophysis, between the two points of attachment with the brain, is almost rectangular and is surrounded by the lateral lobes (fig. 12). The last drawing of the series (fig. 13) shows the rectangular anterior lobe partially surrounded by the lateral lobes, and lying against the ventral surface of the pars intermedia and the cerebral-carotid arteries on either side. The pars intermedia, which in embryos forms a wide mass, closely applied to the ventral surface of the infundibulum and extending between its various branches, is in contact with the upper surface of the anterior lobe.

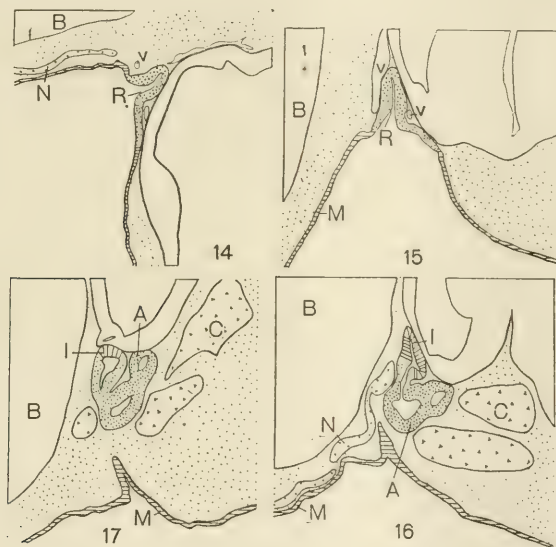
Development in lizards

Young *Lacerta* embryos of 1.6 to 3 mm. in length do not show the relation between the epithelial linings of the pharynx and the head cavities described by Salvi ('12, '02). No distinct hypophyseal angle is present in a 2.8 mm. embryo.

In a 3 mm. embryo (*Lacerta agilis*) a distinct hypophyseal angle of thickened epithelium is present. In a reconstruction of this part, the hypophysis is a wide ridge lying close to the wall of the brain. On either side, near the edge of the ridge, the cerebral carotids lie close to the epithelium. Dorsal to the median part is a large venous channel. Caudal to the ridge a prominent angle of epithelium projects dorsalward beyond the hypophyseal angle.

A wax reconstruction of a 5.0 mm. embryo shows a slight median evagination from the oral roof—Rathke's pouch (fig. 63). The caudal side descends sharply while the cranial side slopes gradually and is continuous with an anterior thickened epi-

thelium. On either side of Rathke's pouch are smaller evaginations, long cranio-caudal ridges—the lateral buds—separated from the pouch by distinct furrows. The opening to the out-pouching is wide (fig. 14), the lumina of the three evaginations forming a common hypophyseal cavity. The carotid arteries lie on either side of the hypophysis, near the lateral lobes. On either side, also, are veins with anastomosing branches, one



Figs. 14 to 17 Median sagittal sections of the hypophyses of lizard embryos. $\times 40$ (M. U. E. C. 565, 576, 563, 564 *Lacerta agilis*). 14, 5 mm.; 15, 9 mm.; 16, 11.6 mm.; 17, 15 mm. (greatest length of coil). A, anterior lobe; I, pars intermedia; R, Rathke's pouch; B, section of brain wall; C, cartilage; M, epithelium of mouth; N, notochord; v, veins.

lying caudal to Rathke's pouch, the other just anterior, in the angle formed by the cranial wall of Rathke's pouch and the anterior thickened arm of epithelium. As Gaupp ('93) has stated, a venous channel thus surrounds Rathke's pouch. Caudal to the pouch, the roof of the pharynx forms a slight angle, Seessel's pouch, less prominent than in the 3 mm. embryo. The notochord comes into contact with the epithelium of this angle.

In a 9 mm. embryo, Rathke's pouch is widened transversely, but is constricted at its base. The lateral lobes extend dorso-laterally from just below the constricted portion (fig. 64). Their bases are slightly anterior to that of Rathke's pouch. The thickened anterior arm of epithelium has formed a medial out-pouching, anterior to Rathke's pouch (fig. 15). A reconstruction of this region shows, from the oral surface, a cup-like depression and a wide medial opening of Rathke's pouch. On either side, just anterior to this, are the two openings of the lateral lobes. Seessel's pouch is inconspicuous.

Both laterally and ventrally the carotids come into contact with the lateral lobes. The vein described in the 5 mm. embryo retains the same relations in this embryo, but the anastomosing branch, caudal to Rathke's pouch fuses to form a large venous channel (fig. 15).

Marked changes have taken place in an 11.6 mm. embryo, where the anterior bud is constricted from the oral roof and the four-lobed hypophysis is attached to the mouth by means of a narrow stalk. Projecting caudo-dorsally to this stalk is an epithelial cord which possibly represents Seessel's pouch. The short, hypophyseal stalk extends dorso-cranially and is attached to the ventro-anterior wall of the anterior bud. The caudal lobe, or Rathke's pouch, is closely applied to the small infundibular outgrowth and has a flattened tip. Rathke's pouch is markedly constricted where it joins the large anterior bud (fig. 16). The latter is closely applied to the floor of the brain. On either side, between these two parts, lie the short, finger-like lateral lobes extending upward, but not in contact with the floor of the brain. Below they open into the cavity of the anterior lobe, which widens to receive them. Caudal to the constricted portion of Rathke's pouch is an oval-shaped mass of cartilage which contains the tip of the notochord. Lateral cartilaginous masses extending anteriorly are continuous with that mentioned above. Dorsal to the anterior end of the lateral masses and cranial to the anterior lobe, is a condensed connective tissue or precartilaginous mass. It projects upward to the brain floor and forward to the optic chiasma. A fibrous band of connective tissue

lies along the posterior surface of Rathke's pouch and extends between the diencephalon and mesencephalon. The carotids are crowded between the lateral cartilages and the lateral lobes.

In a 15 mm. embryo the hypophysis is only slightly larger. The two important changes are the increase in length of the anterior lobe, which has grown dorsalward almost as far as Rathke's pouch (fig. 17), and the lack of development of the lateral lobes. Rathke's pouch, lying below the infundibular outgrowth, has an expanded upper free end and a constricted neck connecting it to the enlarged lower portion of the anterior lobe. The small lateral lobes open into the anterior lobe in front of the opening of Rathke's pouch (fig. 65). The anterior cartilages have the same position as in earlier embryos. On either side of the upper part of the hypophysis are two masses of striated muscle; the carotids lie along the lateral sides of the lateral lobes.

The hypophysis of a 17.5 mm. (total length) *Lacerta muralis* is not as well-developed as the 15 mm. *Lacerta agilis* just described. The lateral buds are relatively larger and open into the anterior bud on either side of the constricted attachment of Rathke's pouch. Venous channels separate them from Rathke's pouch and join by an anastomosing branch in front of the latter.

The lateral buds in a 28 mm. *Lacerta muralis* (H. E. C. 809) are in contact with the brain floor on either side of Rathke's pouch. Their connections with the anterior lobe are smaller than in the specimen described above. The tip of Rathke's pouch is somewhat expanded laterally. The anterior bud has many glandular outgrowths and a small lumen continuous with that of Rathke's pouch.

In a 37 mm. *Lacerta viridis* (H. E. C. 604), two epithelial masses lie imbedded in the wall of the brain. Ventro-medially they extend towards the anterior lobe, but are not in contact with it. The wide anterior lobe, now with many glandular outgrowths extending cranialward, is continuous caudally and dorsally with the flattened tip of Rathke's pouch. The lumen of the latter is flat and extends a short distance into the anterior lobe.

A median sagittal section of an adult *Sceloporus* shows only two parts to the hypophysis; the anterior lobe and the pars intermedia. The anterior lobe, shorter than in turtles, lies close against the brain floor (fig. 47). Its anterior end is oval and the caudal end flattened. The pars intermedia has a residual lumen with a thick roof and a thin floor, the latter, continuous below with the anterior lobe (fig. 47). A series of transverse sections of turtles (figs. 9-13) were drawn at varying distances from the anterior end. The levels at which all transverse sections of reptiles were drawn are shown in table 3. The first drawing of the series of *Sceloporus* (fig. 18) shows the anterior lobe, the floor

TABLE 3

Table showing levels at which the drawings of transverse sections of the hypophyses of various reptiles were taken, using an adult turtle as the standard

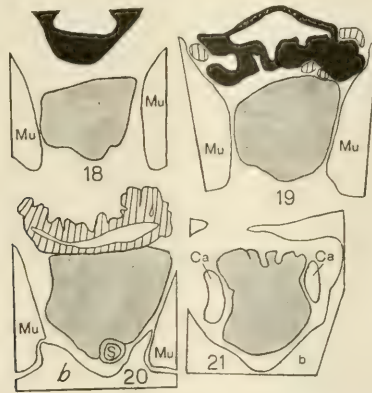
	DISTANCES FROM THE ANTERIOR END OF THE HYPOPHYSIS OF AN ADULT TURTLE				
	0.61 mm.	1.23 mm.	1.91 mm.	2.52 mm.	3.28 mm.
Adult turtle.....	Figs. 9	10	11	12	13
28 mm. turtle.....	Figs. 6		7	8	
Adult lizard.....	Figs. 18		19	20	21
30 mm. alligator.....	Figs. 27	28			29
Adult alligator.....	Figs. 30	31	32	33	34
12 cm. snake embryo.....	Figs. 39			40	41
Adult snake.....	Figs. 42	43			44

of the brain and the muscles at either side; figure 19, the infundibulum and the anterior end of the pars intermedia. In the next figure the pars intermedia with its central cavity and the anterior lobe are shown. In the ventral part of the anterior lobe is a cystic tubule (fig. 20). The relations of the cranium to the hypophysis are seen in figure 20. The caudal end of the anterior lobe is entirely surrounded by bone and the two cerebral carotid arteries lie at either side (fig. 21).

Development in alligators

In the 12 mm. alligator studied, the four parts of the hypophysis described in other reptiles are well formed. The lateral buds are finger-like processes on either side, anterior to Rathke's

pouch. They open caudo-laterally into the anterior bud near the hypophyseal stalk (fig. 68). Rathke's pouch lies against the brain in the region of the undeveloped infundibular evagination (fig. 22). The notochord curves downward and backward and comes into contact with the caudal surface of the pouch. The cerebral carotid arteries lie on either side, caudal to the lateral buds. The floor of the anterior lobe is connected by a wide stalk with the pharyngeal roof. This lobe projects cranially, and its caudal end is continuous with Rathke's pouch.

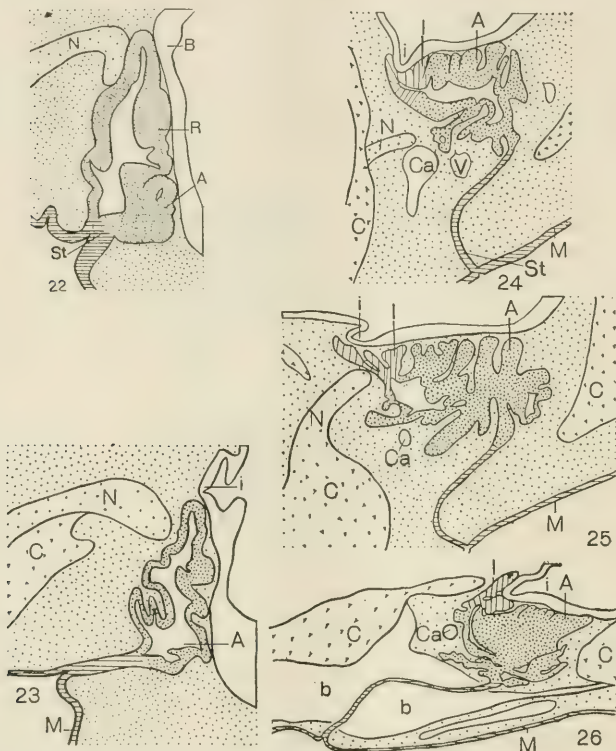


Figs. 18 to 21 Transverse sections of the hypophysis of an adult *Sceloporus* $\times 35$ (W. U. C. 188). Fine stipple, anterior lobe; lined, pars intermedia; black, brain and infundibulum; *b*, basis cranii; *Ca*, carotids; *Mu*, muscles; *s*, remnant (?) of stalk.

Dorsal to the attachments of the lateral buds, are two evaginations from the anterior lobe extending toward the brain. Reese figured these evaginations (Reese '10, fig. 4b, *o''*) but did not follow their further development. The evaginations shown by him in figure 4, *b*, and *c, o'*, correspond to the lateral buds of other reptiles.

In a 19 mm. embryo Rathke's pouch projects dorsally beyond the tip of the notochord. The lateral buds are elongated. The hypophyseal stalk is attached to the floor of the anterior lobe near its cranial end. The stalk arches caudally where it is continuous with the oral roof (fig. 23).

In a 22 mm. embryo, it is evident that the position of the hypophysis has shifted because of the straightening out of the cranial folds. The whole organ has elongated. The tip of the notochord lies caudo-ventral to Rathke's pouch (fig. 24). There



Figs. 22 to 26 Median sagittal sections of alligator embryos. 22, about 12 mm. (R. C., F. 16) $\times 50$; 23, 14 mm. $\times 40$; 24, 22 mm. $\times 40$; 25, 28 mm. $\times 30$; 26, 120 mm. $\times 15$ (W. U. C. 175, 176, 177, 178). A, anterior bud or lobe; I, pars intermedia; R, Rathke's pouch; b, basis cranii; B, brain wall; Ca, anastomosing branch of the carotids; c, cartilage; i, infundibulum; M, epithelium of mouth; N, notochord, St, stalk.

are many outgrowths from the dorsal and anterior walls of the anterior lobe and a few from the floor. Of the latter a very prominent one extends caudally, ending in front of the anastomosing artery of the cerebral carotids. The hypophyseal stalk

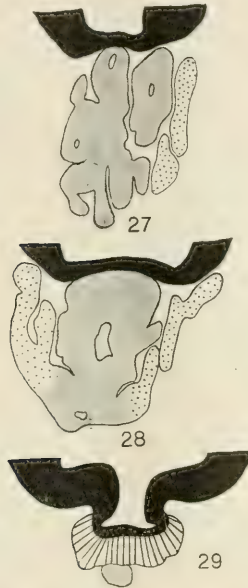
attached to the floor of the anterior lobe projects ventro-caudally and is continuous with the pharyngeal roof. The lateral lobes have many tubular outgrowths at their free ends. The tip of Rathke's pouch has curled about the infundibular outpouching.

The hypophysis in a 28 mm. embryo bears little resemblance to the sac-like outgrowth of the younger embryos (fig. 25). There is a small cavity in the caudal portion of the anterior lobe continuous with that of Rathke's pouch. The remainder of the organ is made up of cords with occasional lumina indicating a tubular development. A prominent caudal tubular evagination of the floor of the anterior lobe extends above, and another below the anastomosing branch of the carotids. Between the former and the tip of Rathke's pouch is the anterior end of the notochord. The hypophyseal stalk extends ventro-caudally from the floor of the anterior lobe. Near its oral end it makes a sharp ventral bend and joins the pharyngeal epithelium.

A series of drawings of the hypophysis of a transversely sectioned embryo, at least 30 mm. in length, is shown in figures 27 to 29. The relations and extent of the anterior lobe and lateral buds can be seen. Lumina are present in some of the glandular outgrowths of the anterior lobe, but the lateral buds are solid. The pars intermedia is ventral to the infundibulum.

In a 12 cm. embryo, Rathke's pouch curls around the dorsal side of the well-developed infundibular evagination (fig. 26). The pouch is constricted in its middle, and its antero-ventral end is continuous with, and forms part of, the anterior lobe. The latter is now one large mass of cords, continuous laterally with cords developed from the lateral buds which extend dorsally and join across the median line. Many cords with free ends project cranialward and caudalward from the floor of the anterior lobe. A caudal cord lies just below the anastomosing branch of the cerebral carotids which is crowded against the caudal surface of the anterior lobe. From about the middle of the ventral floor of the latter lobe the hypophyseal stalk extends caudalward and, passing through the bony basis cranii, is continuous with the oral roof. It still shows a sharp bend ventralward before the oral roof.

Adult alligators of 22.5 cm. to 150 cm. in length were studied. One of these, cut sagittally, shows clearly the parts with which we have to deal in the adult form. The main part of the hypophysis, the anterior lobe, is a short oval mass lying below the infundibular outgrowth (fig. 48). As in late embryos, the pars intermedia forms a layer around the infundibular outpouching. The distal portions of the lateral lobes lie against the floor of the

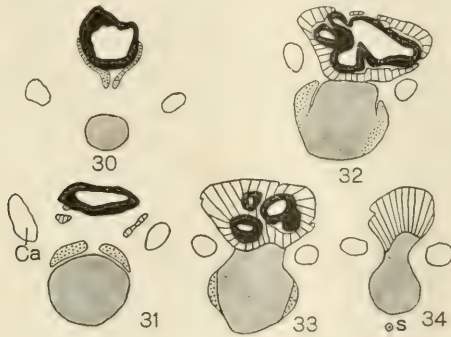


Figs. 27 to 29 Transverse sections of an alligator embryo. $\times 40$ (R. C. 9, possibly over 30 mm. long). Fine stipple, anterior lobe; coarse stipple, lateral lobes; lined, pars intermedia; black, brain and infundibulum.

brain and are connected with the proximal parts by means of small stalks which descend caudalward over the top of the anterior lobe (figs. 30 to 32). The proximal parts lie to either side of the anterior lobe and are continuous ventrally with the caudal part of it (fig. 33). The anterior lobe ends cranially in a blunt apex, separated from the floor of the brain by connective tissue, and caudally often in two blunt points separated by the anastomosing branch of the cerebro-carotid arteries (fig. 48). The upper of the caudal points continues caudalward below the pars

intermedia. A small cystic tubule is often found on the ventral side of the anterior lobe caudal to the lateral lobes (fig. 34). Near its caudal end this tubule becomes solid. Judging from its position and from the fact that the hypophyseal stalk is present in very late embryos this elongated tube is probably the remnant of it.

In a specimen about 22 cm. long, a ventral stalk-like tubule extends caudally, then ventrally and ends blindly in a notch in the floor of the cranium. A strand of connective tissue continues from the blind end of the tubule through a small canal in the



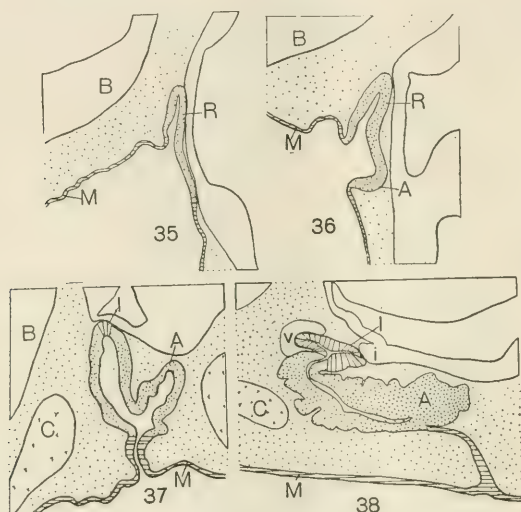
Figs. 30 to 34 Transverse sections of the hypophysis and infundibulum of an adult alligator 47 cm. long. $\times 10$ (W. U. C. 213). Stipples and lines as in figure 27. *Ca*, carotid arteries; *s*, remnant of stalk.

floor of the cranium and turning to the right ends under a granular epithelium lining a cavity in the cranial floor. This stalk, about 75 micra in length, is very much shorter than the hypophyseal stalk in a 12 cm. embryo.

Development in snakes

Snake embryos (*Vipera verus*) 3 mm. long have a median evagination resembling in general shape and position Rathke's pouch in other reptiles. Embryos 5 mm. long have a very much longer median pouch and at either side definite lateral ridges (fig. 66). The furrow separating these ridges from the median pouch never becomes as prominent as in turtle and lizard embryos. A median sagittal section of a 7 mm. Tro-

pidonotus natrix embryo shows a well-developed Rathke's pouch continuous with an anterior, thickened, slightly evaginated epithelium, the anlage of the anterior bud (fig. 35). In an embryo 9 mm. long (fig. 36) there is a definite constriction of the hypophysis from the oral roof. The walls of Rathke's pouch are thickened and the lumen is continuous with that of the well-marked evagination of the anterior lobe. The lateral buds



Figs. 35 to 38 Median sagittal sections of the hypophyseal region of snake embryos. $\times 40$ (M. U. E. C. 570, —, 571 *Tropidonotus natrix*). 35, 6.85 mm.; 36, 9 mm.; 37, 12 mm. (diameter of coil); 38, 10 cm. (total length). A, anterior bud or lobe; I, pars intermedia; R, Rathke's pouch; B, brain wall; C, cartilage; i, infundibulum, M, epithelium of mouth and stalk; v, veins.

are small and not sharply separated from the caudal end of the anterior evagination.

In some *Tropidonotus* and *Eutaenia* embryos, 9.5 to 10.5 mm. long, the lateral buds, which disappear in later embryos, are still present. In an embryo (*Eutaenia*) 10 mm. in length there are two very small evaginations, probably rudiments of the lateral buds.

A reconstruction of a 12 mm. embryo (*Tropidonotus natrix*) shows a well developed Rathke's pouch joining, at an acute angle,

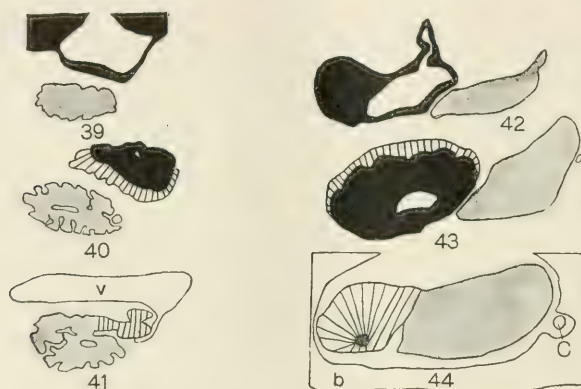
the anterior lobe (fig. 67). The former is wide and somewhat concave where the slight infundibular outgrowth lies in contact with it. The anterior lobe is also wide and almost comes into contact with the brain floor in front of the infundibulum. Very small projections at the base of the anterior lobe, more marked on the right side than on the left, are the only remaining evidence of the lateral buds. The wide ventral end of Rathke's pouch somewhat overhangs the small hypophyseal stalk which is attached to the floor of the anterior lobe. The stalk is arched slightly forward. From both surfaces of the anterior lobe there are many small evaginations, not evident in the reconstruction (fig. 67), but shown in the figure of a sagittal section (fig. 37).

A median sagittal section of a 10 cm. *Tropidonotus* embryo shows distinct growth of all parts and particularly of the anterior lobe (fig. 38). Most marked, however, is the apparent straightening out of the hypophysis and the obliteration of the early acute angle between Rathke's pouch and the anterior bud. The anterior lobe, now elongated cranio-caudally, has a very irregular outer surface, due to the great development of tubules. The solid hypophyseal stalk, extending forward and downward, is attached to the hypophysis near its anterior end and is continuous with the oral roof. The pars intermedia is a large well-developed outgrowth from the superior end of Rathke's pouch. Caudally it is surrounded by a large venous channel and anteriorly it comes into contact with the tip of the small infundibular evagination. The narrow lumen of the pars intermedia is continuous with that of the anterior lobe. The lateral buds have disappeared and there is no evidence of any structure having developed from them. A thin connective tissue layer joining the tips of the cartilages forming the floor of the chondrocranium, lies in close contact with the ventral surface of the hypophysis. A similar layer appears to anchor the dorsal surface of the anterior lobe to the floor of the brain.

A series of cross sections of a 12 cm. *Eutaenia sirtalis* embryo shows the relations of the hypophysis to the brain and the relative sizes of its parts. The first drawing (fig. 39) shows the anterior lobe with its irregular surface lying to the right of the

median line. The second shows a slit-like lumen in the anterior lobe, and the pars intermedia attached to the floor of the brain near the infundibulum (fig. 40). The entire anterior lobe lies somewhat to the left of the mid-sagittal line of the brain.

Heterodon platyrhinos, 40 to 70 cm. in length, and *Eutaenia sirtalis* were used for the study of the adult snake hypophysis. In a median sagittal section of the latter (fig. 46) the infundibulum is closely surrounded by a relatively thick pars intermedia, which in turn is partially encircled by the anterior lobe. The



Figs. 39 to 41 Transverse sections of the hypophysis and brain floor of a 12 cm. (total length) *Eutaenia sirtalis* embryo. $\times 40$ (W. U. C. 48). Fine stipple, anterior lobe; lined, pars intermedia; black, brain and infundibulum. *v*, vein.

Figs. 42 to 44 Transverse sections of the hypophysis of an adult adder (*Heterodon platyrhinos*). $\times 20$ (W. U. C. 194). *b*, basis cranii; *C*, carotid artery; stipples and lines as in figure 39.

latter is a rather thin elongated mass with a small rounded anterior end. The dorsal surface of the anterior lobe lies against the floor of the brain for a short distance. Between the two, at the anterior end, a thin layer of connective tissue is interposed. In a sagittal section to the right of the median line, the anterior lobe is thicker than in the section just described.

A series of transverse sections of *Heterodon* show that in this form, as in *Eutaenia*, the anterior lobe lies to the right of the median line. The first section (fig. 42) of the series shows a wide anterior lobe, the left side of which is near the brain floor. The

right side lies close to the wall of the pituitary fossa. In the next section drawn (fig. 43) the dorsal surface of the large infundibulum is in contact with the thin pars intermedia. The large anterior lobe lies to the right of instead of ventral to the pars intermedia and the infundibulum. Since the same relations are present in late snake embryos, this is probably not an artefact as might at first appear. The right side of the anterior lobe lies near the bony wall of the pituitary fossa in the lower angle of which is seen the cerebro-carotid artery. The left side of the pars intermedia lies against the cranial floor. The caudal end of the hypophysis is surrounded by the wall of the pituitary fossa (fig. 44).

Discussion

In reptiles, the first appearance of the hypophysis is a thickened area of the epithelium of the oral roof and then a single evagination of the latter. As Staderini ('03) has pointed out, Gaupp ('93) was probably mistaken in describing a primary tri-lobed hypophyseal anlage in lizards. Earlier investigators, with the exception of Gaupp, believed the anlage of the hypophysis to be a single evagination, and the tri-lobed condition to be of secondary origin, and recent investigators of the hypophysis of vertebrates also describe a single hypophyseal anlage. The single evagination in turtles is triangular in outline and two lateral buds are constricted from its sides. The lateral buds develop relatively earlier in lizards, but as I have attempted to show, are probably of secondary origin. In mammals and birds (Tilney '13) and in pig (Miller '16) the lateral buds develop relatively very much later than in reptiles.

Salvi ('02) has attempted to prove that the lateral buds are connected with the head cavities. That this is not true of turtles is apparent from a study of my young embryos, in which, in sagittal sections, the canal joining the first head cavities is caudal to the hypophysis. A reconstruction of another young turtle embryo also shows this dorso-caudal canal. Oppel ('90) and Johnson ('13) figure no such relation as Salvi has described. In turtles, Johnson figured the canal joining the head cavities dorso-caudal to the hypophysis and distinctly separate from it.

In a reconstruction of the hypophysis of a very young turtle there are indications that furrows separating the lateral buds from Rathke's pouch begin on the cranial side. Furrows first appear at the same place in the separation of the inferior lobes from Rathke's pouch in *Squalus*. (Baumgartner '15).

In the later development of the lateral buds in turtles, the tips grow forward and form distinct distal portions. In 28 mm. embryos these have approached each other across the dorsal side of the anterior lobe, and in adults, form a thin layer closely applied to the floor of the brain. Gisi ('07) believed the same thing occurs in *Sphenodon* and said: "Wahrscheinlich ist diese Pars terminalis der Hypophyse das Endproduct der seitlichen Knospen an den früheren Embryonalstadien." The development from the lateral buds of the tongue-like process extending below the brain is similar to that observed by Herring ('08) and carefully described as the *pars tuberalis* in mammals and birds by Tilney ('13). Joris ('07) undoubtedly saw the same structures as isolated masses near the brain floor in mammals.²

The developing proximal portions of the lateral buds surround the anterior lobe, thus forming a thin cortical layer about the middle of this lobe. Gisi did not note this in *Sphenodon* nor did Tilney describe it in mammals. Miller's ('16) description of a somewhat similar layer in the pig indicates that it may have been overlooked.

The development of the hypophysis in alligators is similar to that in turtles. Although Reese ('10) did not find lateral lobes, a wax reconstruction of the alligator hypophysis shows them to be present (fig. 68). During development they extend anteriorly and dorsally. In an adult specimen 47.5 cm. long the distal portions of the lateral buds are not continuous across the median line as in adult turtles, while in an adult 150 cm. in length the distal portions are fused and form a single tongue-like *pars tuberalis*. It would seem, therefore, that there is some

² Woerdemann ('14) has given a full review of the literature on the various portions of the embryonic and adult hypophysis. This author saw, in the 'lobus chiasmaticus' and 'lobus praemammillaris' of Staderini ('08 and '09) and also in the 'lobule de la tige' of Joris ('07), a structure homologous with Tilney's ('13) *pars tuberalis*. With this view I am in agreement.

growth in the hypophysis, or at least of this part, during adult life. Increase of glandular growth during adult life in *Acanthias* has also been noted. The band-like proximal portions of the lateral buds curve backward around the sides of the anterior lobe to near its caudal end.

In a median sagittal section of a 3 mm. lizard embryo there is a well marked hypophyseal angle. In the next embryo described Rathke's pouch and the lateral buds are well developed, the latter being separated from Rathke's pouch by wide venous sinuses as described by Gaupp ('93). In 15 mm. *Lacerta agilis* embryos the lateral buds appear as two small, blunt evaginations. Their later history in *Lacerta agilis* could not be followed. However, in a transversely sectioned series of *Lacerta muralis*, 28 mm. long (total length, H. E. C. 809) the lateral buds are closely applied to the floor of the brain on either side of Rathke's pouch. In a specimen of *Lacerta viridis* 37 mm. in length, two masses of cells, apparently the lateral buds, are imbedded in the meninges and are completely isolated from the hypophysis. It can not be said that these masses are degenerating. In a limited series of *Hemidactylus* adults, such masses of cells were not observed and in adult *Sceloporus* they are entirely absent. In *Lacerta ocellata*, *agilis* and *muralis*, Haller ('96) and Gisi ('07) found nothing corresponding to a *pars tuberalis*, although Gisi described a '*pars terminalis*' in *Chamaeleon*. In late *Lacerta* embryos Gaupp found that the lateral buds become isolated masses imbedded in the meninges. Staderini ('03) and Bruni ('14) described a similar development in *Gongylus* embryos. Later ('05) Staderini described isolated masses imbedded in the brain of adult *Gongylus* although Orrù ('00) does not mention them in either embryos or adults of this form. It appears then that the lateral buds persist only in *Gongylus*, *Chamaeleon*, and possibly in *Lacerta*, although the latter needs confirmation. Stendell ('14) stated that the tip of the anterior lobe in *Lacerta vivipara* forms the '*zungenförmigen Fortsatz*.' This does not agree with my observations on the development of the *pars tuberalis* in alligators and turtles or with those of Gisi ('07, *Sphenodon*) and Tilney ('13, cat, bird).

In the snakes studied the lateral buds do not persist. Young embryos of *Eutaenia sirtalis* and *Tropidonotus natrix* show well-defined but not distinctly separated lateral buds. A *Tropidonotus* 12 mm. long (diameter of coils) shows only a questionable bud, while in 10 and 12 cm. embryos the lateral buds have entirely disappeared as is the case in adult garter snakes. They are not present in adult adders. Tilney ('11) apparently saw no tongue-like process in *Eutaenia sirtalis*.

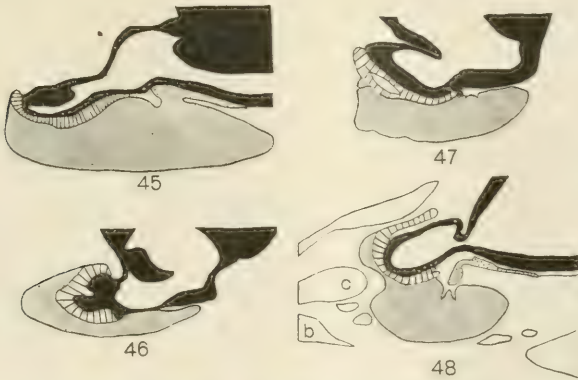
Briefly, then, the lateral buds persist and form a *pars tuberalis* and cortical zone about the anterior lobe in turtles (*Aromochelys odorata* and *Chrysemys marginata*); a tongue-like process and spiral band around the anterior lobe in alligators; a *pars terminalis* in *Sphenodon* (Gisi '07); disappear in lizards and snakes except in *Gongylus* (Staderini '05 and Bruni '14), *Chamaeleon* (Gisi '07) and possibly *Lacerta agilis* and *Anguis fragilis* (Gaupp '93).

The third outpouching from the oral roof, Gaupp's 'vordere Knospe' together with the ventral part of Rathke's pouch, gives rise to the adult anterior lobe, with the exception of its cortical layer in turtles and alligators. I can see no reason for dividing the anterior evagination in reptiles into 'Vorraum und Mittelraum' as Woerdemann ('13) has done.

The shape of the anterior lobe in adult reptiles is shown in the drawings of median sagittal sections (figs. 45-48). In turtles its length is about three times its depth. Gisi's ('07) measurements for *Emys lutaria* are four to one. In alligators and snakes, it is about two to one and in lizards about three to one. In snakes the anterior lobe lies somewhat lateral to the infundibular outgrowth, so that a median sagittal section of the brain does not show the greatest depth of the anterior lobe. The *pars intermedia* in the forms studied is a thin layer interposed between the infundibular outgrowth and the anterior lobe. The latter, in snakes and alligators, lies against the curved, ventral surface of the *pars intermedia*, and in snakes, alligators, and some turtles, it extends dorsally over the sides and caudal end of the infundibulum. Herring ('13) figures this part in *Testudo graeca* as extending forward from between the *pars nervosa* and

pars glandularis (anterior lobe), forming a thick layer over the anterior end of the latter. In the turtles and alligators studied, the pars intermedia is not continuous anteriorly with the pars tuberalis as Gisi described for *Testudo graeca*. In snakes only does the anterior lobe come into contact with the floor of the brain (fig. 46).

The dorsal tip of Rathke's pouch in 10 mm. turtles proliferates, and in 14 mm. embryos is somewhat constricted from the remainder of the pouch (fig. 4). This dorsal tip, not the entire



Figs. 45 to 48 Median sagittal sections of brains of reptiles showing infundibulum and hypophysis. 45, turtle $\times 10$; 46, snake $\times 13$; 47, lizard $\times 35$; 48, alligator $\times 13$ (W. U. C. 202, 186, 190, 212). Fine stipple, anterior lobe; coarse stipple, parts derived from lateral buds; lined, pars intermedia; black, brain and infundibulum. *b*, basis cranii; *c*, anastomosing branch of carotids.

pouch, gives rise to the pars intermedia (Tilney's pars infundibularis, Sterzi's chromophobic). A similar development was described by Bruni ('14) in *Gongylus* and by the writer ('15) in *Acanthias*. Bruni states: "Dei tre lobi il *medio*, destinato a mettersi in rapporto col processo infundibulare . . . , origina come un diverticolo dalla parte più distale della parete rostrale della tasca di Rathke. . . . " (p. 186). A distinct constriction of Rathke's pouch where it joins the anterior bud is evident in young turtle embryos. In 9 mm. embryos the constriction is less evident than in younger, and in 17 mm. embryos it is no longer apparent. Woerdemann ('14) noted

this early constriction and realized that the later constriction separating the pars intermedia from the anterior lobe is not the same. In adult *Sceloporus*, at least, the pars intermedia is never completely constricted from the anterior lobe.

The hypophysis in adult turtles and lizards is ventral to the third ventricle, although in turtles the infundibular outgrowth is long. In alligators the infundibulum is long and the short hypophysis lies ventral to it. The infundibulum in snakes is short but the hypophysis extends relatively more caudalward.

In the material studied, only adult *Sceloporus* show a residual lumen and this is in the pars intermedia. An adult *Hemidactylus* shows no cavity. Müller ('70) described a cavity in the hypophysis of *Testudo*. Haller ('96) termed the pars intermedia 'Sammelschlauch' and figured tubules of the posterior portion of the main lobe emptying into its lumen. Sterzi ('04) believed this to be incorrect as he found no residual lumen in lizards and turtles. Gisi ('07) described a lumen filled with a coagulum in *Testudo graeca*. Tilney ('11) mentioned a lumen in the hypophysis in turtles, snakes and alligators. Herring ('13) figures one between the pars intermedia and the pars glandularis of turtles, while Stendell ('13 and '14) figures one in the 'Zwischenlappen' of *Lacerta* and one between the 'Zwischenlappen' and 'Hauptlappen' in *Emys*, although he states that its position and occurrence varies in the Sauropsida. In turtles I have found that, even with very careful preparation of specimens, occasionally some of the pars intermedia is lost from the sections. Because of the irregular walls, a lumen sometimes present between the pars intermedia and the anterior lobe, is not thought to be a residual lumen but an artefact as Sterzi ('04) believed. A lumen which might persist in the adult would be in the pars intermedia because of the manner of its development.

A well-developed stalk, formed by a constriction of the hypophyseal anlage from the roof of the mouth is present in 6.4 mm. turtles, 9 mm. lizards and snakes, and 12 mm. alligators. The stalk remains short in turtles and lizards, is slightly longer in snakes and becomes very long in alligators. It has degenerated in 17 mm. turtles, except for a small cystic dilatation at

the hypophyseal end. In lizards it is still present, although very small in 28 mm. embryos. In both of these animals the caudal side of the stalk is convex. Gaupp stated that while the stalk is present in a 47 mm. *Anguis fragilis*, it has disappeared in the 60 mm. stage. Melcher ('99) found that the stalk disappeared at about the 21 mm. stage in *Gecko* and Gisi ('07) in 20 mm. *Sphenodon*, although she found a strand of connective tissue indicating the position of the stalk in 28 mm. embryos. Bruni ('13) described a remnant of the stalk in an adult *Gongylus* and Staderini ('05) a foramen in the cranium for the remnant of a stalk also in an adult. In young snake embryos the stalk is convex caudally as in turtles and lizards. In a 10 mm. embryo it arches forward at the hypophyseal end and has disappeared in 12 cm. embryos.

Reese ('10) stated that the hypophyseal stalk had degenerated in its middle part in a 6 cm. alligator embryo, and that only a small part at the hypophyseal end remained in a 13 cm. embryo. In the 12 cm. embryo here described, the entire stalk is present (fig. 26). It is long and S-shaped as described by Reese, arching caudalward and ventralward from the hypophysis and extending in the direction opposite to that found in snakes.

Lizards have a small mass of epithelium projecting dorso-caudally from the stalk where it is attached to the oral roof. The close relationship between this mass (Seessel's pouch) and the hypophyseal stalk, found only in this group of reptiles, has apparently not been observed by other investigators studying *Lacerta agilis*. It is similar to that observed by Bruni ('13) in chick. Gaupp ('93) figured Seessel's pouch further caudalward in a 27 mm. *Lacerta*.

In figures 1 to 5 of young turtle embryos, there is a relative caudalward migration of the attachment of the stalk to the hypophysis, while in alligators (figs. 22-26) and snakes (figs. 35-38) the attachment remains near the anterior end of the hypophysis. In an alligator embryo 12 cm. long, however, the attachment is more caudal than in younger ones. This may be due to the growth of the hypophysis cranialward, or to the interference of the developing cranial floor. That there is

an actual shifting of the hypophyseal stalk in alligators is seen in figure 51 where the point of attachment to the oral roof has been taken as a fixed point.

As described by Gaupp, Bruni and others, in all forms the stalk is attached to the anterior lobe. In older turtle embryos it extends from the ventral surface of the anterior lobe near its caudal end; in lizards from somewhat nearer the anterior end (described by Bruni in *Gongylus*); and in snakes and alligators close to the anterior end.

A long tubule, extending caudally from the ventral surface of the hypophysis in an adult alligator 47.5 cm. in length, as well as one in a 22 cm. embryo extending through the cranium almost to the epithelial lining of one of the nose cavities, is undoubtedly the remnant of the stalk. In these adult specimens as well as in late embryos this stalk extends caudalward from near the caudal end of the anterior lobe.

There is reason for thinking that some of the tubules described in some of the turtles and lizards are also remnants of the stalks. In a young turtle a tubule extends forward from about the middle of the hypophysis. If this be the remnant of the stalk, it may be that the shifting of its attachment has been brought about by the growth of the cranium, or, to a late caudalward shifting of the hypophysis. In an adult *Sceloporus* a similar cystic tubule extends along the ventral surface of the hypophysis for some distance. It is possible, of course, that occasionally the stalk will persist at least until adult life. This possibility, together with the position and appearance of the tubules make it probable that the structures described are hypophyseal stalks or remnants of stalks, although they may be dilated tubules. There is no evidence in any of the specimens of any proliferation from the pharyngeal end of the stalk forming a pharyngeal hypophysis such as has been described in some mammals.

Saint Remy ('96) has located the anterior end of the notochord in various Amniota. In reptiles he found it attached to the entoderm between Rathke's and Seessel's pouches. He described ascending and descending limbs of the anterior end of

the chorda and stated that the latter degenerates or is transformed into other tissue.

In young turtle embryos the tip of the notochord frequently comes into contact with a small tubular projection from the caudal surface of the hypophysis near the hypophyseal stalk. Up to the 9.4 mm. stage the notochord ends close to the hypophysis. Later the two are separated, either by a cranialward growth of the hypophysis or by degeneration of the anterior end of the chorda.

In lizard embryos 3 mm. in length the hypophyseal angle is distinctly caudal and ventral, but close to the tip of the notochord. In 5 mm. embryos the tip of Rathke's pouch is anterior to the tip of the chorda and approximately on the same horizontal plane. In embryos 15 mm. in length the chorda is still close to the hypophysis, so that the notochord and surrounding cartilages sometimes cause a depression in the caudal hypophyseal surface. Later the hypophysis is more dorsal and in the same transverse plane.

The anterior end of the chorda in an alligator 12 mm. long is nearer the dorsal part of Rathke's pouch than it is in other reptiles. Later, this part of the pouch, having grown distinctly dorsalward, projects anterior to the tip of the chorda. The notochord ends directly dorsal to the anastomosing branch of the carotids. In a 28 mm. embryo it is still close to the hypophysis, though relatively further ventral. In young alligators, as in turtles, the notochord and its surrounding cartilages depress the caudal surface of the hypophysis. The anterior bud, at first ventral and slightly caudal to the end of the chorda is later anterior to it and in about the same horizontal plane, (figs. 22-26). There is then, in alligators as in pig (Woerdemann '13), a ventral migration of the end of the chorda in its relation to the hypophysis.

In snake embryos the anterior end of the chorda degenerates very early and it is difficult to trace its anterior portion. In one specimen it showed attachment to Seessel's pouch.

In *Aromochelys*, *Chrysemys*, *Lacerta* and alligator, the notochord may come into contact with the hypophysis, but in none

of the reptiles are they continuous, nor does the notochord contribute in the formation of the hypophysis. Recently these structures were again described as being continuous in pig by Woerdemann ('13) and Miller ('16) and in chick by Atwell ('15).³

One turtle embryo shows a cavity in the anterior dilated end of the notochord. Saint Remy found no such cavity in the material he studied and believed that, if such a cavity occurred, it had nothing to do with the formation of a 'Gaumentasche' described by Selenka in opossum. In the pig, Miller ('16) described a part of the notochord becoming detached from the remainder, migrating anteriorly, and forming the medullary part of the anterior lobe of the hypophysis. This is not the case in reptiles. Saint Remy's 'branche descendante' of the notochord, lying in contact with the entoderm was observed in some turtle embryos.

A series of drawings to show the shifting of the hypophysis with relation to the notochord and to its position in the body in lizards and alligators was made after the method described in a previous paper for *Acanthias* ('15). A base line was drawn from the anterior end of the chorda to its axis at the level of the first spinal nerve. The branch of the notochord extending forward to the hypophysis probably corresponds to the slightly enlarged anterior bud of the chorda proper as described by Saint-Remy. The anterior end of the bud is one of the points chosen for the base line of the figures. The caudalward shifting of the notochord with relation to the hypophysis may be due, as Saint-Remy stated, to degeneration. This would prove an objection to using it as an end of the base line since the rate of degeneration probably varies in the different embryos. However, this does not vitiate the general conclusions which may be drawn from the figures.

The anterior lobe, which at first is ventral to the tip of Rathke's pouch, shifts forward and dorsalward in alligators (fig. 50), and dorsalward in lizards (fig. 49) until they are on the same hori-

³ Atwell ('15) has carefully reviewed the literature on the relations of the anterior end of the chorda, and has observed an occasional connection between this and Rathke's pouch in rabbits, and a constant connection in the chick.

zontal plane. The dorsal tip of Rathke's pouch migrates dorsalward and caudalward in lizards and in turtles between the 4.6 and 6.4 mm. stage, and dorsalward and forward in alligators (fig. 50). The shifting of the axis of the hypophysis as shown in figures 49 and 50 is forward and dorsalward as was observed in *Acanthias* (Baumgartner '14).

Bruni ('14) approached the question of the shifting of the stalk and hypophysis from another angle. The writer, for the



Fig. 49 Drawings of median sagittal sections of the hypophyses of lizard embryos (M. U. E. C. 566, 565, 576, 564, 563). A, 3 mm.; B, 5 mm.; C, 9 mm.; D, 11.6 mm.; E, 15 mm. (length of coil); *x-y*, base line.

sake of comparison, made a series of drawings of alligator embryos by Bruni's method (fig. 51). Taking the point of attachment of the stalk to the pharyngeal roof as the center of a circle, an arc is drawn from a caudal point in the pharyngeal roof to an anterior one. Using the center of the circle and the arc as guide lines, and making the distance between the anterior ends of the pharynx at the arc equal to that at the posterior ends, drawings of different embryos were superimposed. Outlines of the hypophyses are also included in the figure. It is to be remem-

bered, however, that the different drawings were not made at the same magnification.

Bruni found that in reptiles, birds, and mammals the axis of the hypophysis and hypophyseal stalk shifts from a horizontal to a perpendicular plane and then back again to a near-horizontal. Figure 51, of a series of alligator hypophyses, shows the axis of the stalk of the intermediate stage, anterior to that of either younger or older stages. It would seem that the length

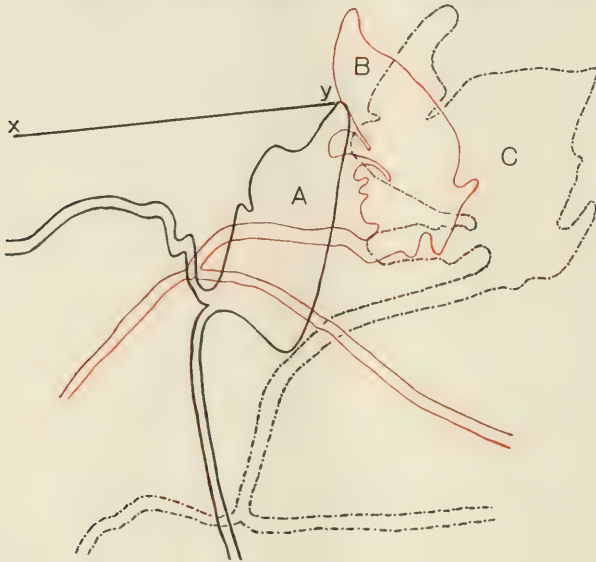


Fig. 50 Drawings of median sagittal sections of the hypophyses of alligator embryos. A, 12 mm. (R. C. 27); B, 22 mm.; C, 120 mm. (W. U. C. 176, 178;) $x-y$, base line.

of the radius of the arc would affect the amount of shifting shown. An interesting comparison between the two methods is made possible by adding the outline of the hypophysis to the method described by Bruni. The latter shows that in young alligator embryos 12 and 22 mm. in length there is a marked forward migration of the entire structure. In the later embryo, 120 mm. there is a dorsal shifting from the position in the intermediate stage (22 mm.). By the first method (fig. 50) the earlier shifting was dorsalward and the latter forward.

Inasmuch as my results for alligators by Bruni's method did not bear out Bruni's conclusions for amniotes, his method was applied to turtles and lizards. The stalk of a 14.5 mm. turtle is slightly caudal to that of a 9.2 mm. embryo. The hypophysis of a 9.2 mm. turtle is dorsal to that of the others. In lizards the hypophyseal stalk of a 15 mm. embryo is caudal to the 3 and 9 mm. stages and the hypophysis more dorsal. The caudalward shifting of the axis of the hypophyseal stalk in turtle and lizard embryos is similar to that described by Bruni in *Gongylus*.

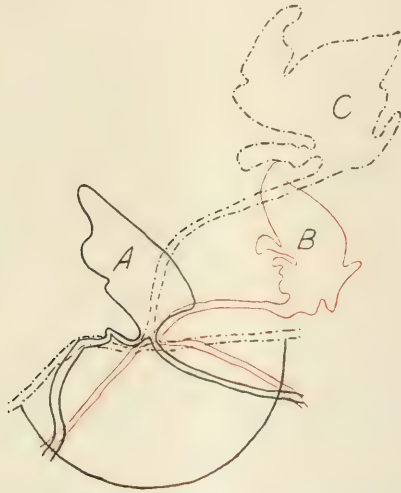


Fig. 51 Drawings of hypophyses of the same alligator embryos as in figure 50. A, 12 mm. (R. C. 27); B, 22 mm.; C, 120 mm. (W. U. C. 176, 178); *x-y*, base line.

It is possible that older embryos, in which the stalk has disappeared, might show a later cranial shifting.

HISTOLOGY OF THE HYPOPHYSIS

All of the adult animals studied were obtained between the months of October and May, except a few lizards which were collected personally. These specimens, with the exception of the alligator, were killed at once and were fixed immediately in 10 per cent formalin or in Zenker-formalin. In the following description the various stains used are indicated.

Turtles

The pars intermedia, surrounding the infundibulum (pars infundibularis of Tilney '13) is composed of laminae of cells. In front there is a single layer which caudally becomes irregular and folded, extending between the various infundibular branches. More caudally there are several laminae, separated from each other by connective tissue and capillaries. They are also separated from the infundibulum and anterior lobe. Figure 56 is a cross section of this lobe and the infundibular wall, showing the arrangement and foldings of the laminae. The cells are columnar. The non-granular cytoplasm stains lightly with eosin. Cell membranes are not always clearly defined, although the appearance is not that of a syncytium. The oval or flattened nuclei are usually placed lengthwise near the center of the cells, so that there is a cytoplasmic zone along either edge of the lamina. The zone in contact with the infundibulum is somewhat wider than those found elsewhere. The nuclei have a very fine chromatin network and usually one, sometimes two or more, prominent nucleoli.

As previously stated, the middle part of the anterior lobe is surrounded by a thin cortical layer derived from the lateral buds. The anterior lobe, with the exception of this zone, is composed of tubules and flattened cords of cells, irregularly arranged as described by Sterzi ('04). Haller ('96) figured these cords running lengthwise. The lumina of the tubules occasionally contain a colloid-like substance. The cells are columnar or polyhedral, with centrally placed nuclei (fig. 52), and take the stain more readily than those of the pars intermedia. Some of the cells stain more deeply than others in the same cords or tubules. This is true of sections stained with eosin-methylene blue and with Giemsa. In sections stained with Mallory's phosphotungstic acid haematoxylin, some of the cells take a purplish color while others are almost colorless. With iron haematoxylin some stain even darker than the nuclei. Again, sometimes all of the cells in one section of a tubule or cord stain deeply, the peripheral cytoplasm as well as that next the lumen retaining the dye.

Presumably it is the minute secretory granules which stain thus intensely. The nuclei, although sometimes indented, are more nearly spherical than those of the pars intermedia. The chromatin network is very light and at least one darkly-staining nucleolus is always present. Often the nuclei of those cells in which the cytoplasm has retained the haematoxylin, particularly phosphotungstic haematoxylin, appear as clear spaces, except for the dark nucleolus. In the clear cells the nuclei may stain very lightly. A few stain intensely with Mallory's phosphotungstic or with iron haematoxylin. Occasional dilated tubules with walls of a flattened epithelium and containing degenerated cells are found. These tubules may be 100 micra long and 50 micra in diameter. Their lumina also contain a colloid-like substance.

A description of the pars tuberalis includes the thin cortical zone of the anterior lobe, both because of its development and because of its histologic appearance. They are composed of narrow strands of cells which are seldom tubular in arrangement, although they may occasionally form solid cords. The strands and cords of the cortical layer are closely applied to those of the anterior lobe, and occasionally extend a short distance into the latter. In many cases they appear to be continuous with those of the anterior lobe. A large amount of connective tissue surrounds the pars tuberalis. The cells are small polyhedrals and stain, like those of the pars intermedia, a light pink (fig. 52). The small nuclei are round and contain a distinct nucleolus. In the cortical zone, as in the main part of the anterior lobe, some nuclei stain very deeply, while only the nucleolus stains in others. In staining reaction the cells of the pars tuberalis and cortical zone appear more like the cells of the pars intermedia than like those of the anterior lobe.

Lizards

In adult *Sceloporus*, there is, as already noted, a central residual lumen in the pars intermedia. The roof of the lumen is composed of a lamina two or three cells deep. A wide periph-

eral zone of cytoplasm lies along the infundibulum and here cell walls are evident. This zone stains lightly with eosin and a cytoplasmic reticulum is apparent. The thin cytoplasmic border of the lumen stains somewhat deeply with eosin so that cell walls, except for a cuticular border, are indistinct. There are two or three rows of nuclei. The upper, spherical nuclei have densely staining membranes and a rather dense chromatin network; the lower row are darker staining, smaller and more crowded. A definite nucleolus is present. The floor of the lumen, although varying in thickness, is about half as thick as the roof. A ventral projection from the floor, near its caudal end (fig. 47) is continuous with the cords of the anterior lobe. The peripheral cytoplasm of the floor around this projection stains intensely, like that of the cords of the anterior lobe, the peripheral cytoplasm of the remainder of the floor staining like the roof. A central cytoplasmic zone is hardly perceptible. The nuclei are arranged in two or three interlocking rows, of which the outer are spherical and the inner often flattened against the surface of the lumen. At either end of the lumen the pars intermedia appears as a single lamina with peripheral cytoplasmic zones and centrally-placed nuclei. One lizard shows very many blue-staining, colloid-like masses (eosin-methylene blue), some as large as nuclei, grouped or isolated in the cells of this part. In the lumen is a reticular coagulum.

The anterior lobe is composed of branching and anastomosing columns with, rarely, an acinar arrangement. The cells are columnar or polyhedral and in their grouping around sinusoids they often give the appearance of an acinus in which the sinusoidal lumen appears as the lumen of the tubule. The distinct peripheral cytoplasmic zone is granular and stains deeply with eosin. The nuclei are spherical and slightly smaller than those of the pars intermedia. A large nucleolus is present and especially prominent in eosin-methylene blue preparations. Occasional cyst-like tubules are present in the anterior lobe. In one specimen a ventral tubule, possibly a remnant of the hypophyseal stalk, is lined by a columnar epithelium having spherical nuclei situated near the base of the cells and tall cilia on the free sur-

face. Some of the cells (eosin-methylene blue) are filled with large blue-staining granules, others with bluish-tinted homogeneous masses, which lie partly in the lumen. In the lumen, also, are large round vacuolated cells and a small amount of blue-staining reticular substance. The spherical nuclei stain homogeneously or show one or two large chromatin masses.

Snakes

In adult garter snakes and adders the pars intermedia, which almost surrounds the infundibular outgrowth, is formed of a thick layer of cells which occasionally dip into the infundibulum. On the outer surface is a very narrow cytoplasmic zone which takes eosin very lightly. Cell membranes are indistinct. The nuclei are spherical, with a light chromatin network.

The anterior lobe, as in *Sceloporus*, is formed of columns or cords which have an outer, narrow, finely-granular rim of cytoplasm in which some cells stain a brilliant red with eosin. Because of the differential staining, it can be seen that the cells are columnar or polyhedral in shape, although cell membranes are not well defined. There are two or three rows of centrally-placed, spherical or oval, light-staining nuclei, usually containing one nucleolus. Many bluish-staining masses, about as large as the nucleoli, are found in some of the reddish-tinted cells.

Alligators

A specimen about 150 cm. in length, fixed in Zenker-formalin, proved to be the best for histologic study. This animal was received in December and kept in a tank for a few days during which time he was frequently disturbed. The sections varied in thickness from 5 to 10 micra and various stains were used.

The pars intermedia is relatively thicker than in smaller specimens. Caudally, the cells are arranged in lamellae which branch anteriorly into irregular cords and completely surround all branches of the infundibulum. The cells are irregular in shape and there is a definite outer zone of cytoplasm. The cytoplasm of centrally-placed nuclei extend between peripheral cells to the

surface of the column. Thus the appearance is that of a pseudo-stratified columnar epithelium in which all cells come to the periphery. The cytoplasm at the center of the columns frequently appears vacuolated and shrunken around the nuclei, leaving irregular spaces. This cytoplasm stains lightly with eosin or orange G. Occasional small areas, the peripheral parts of some cells, stain more intensely. Small, round, homogeneously-staining masses, varying in size, are found grouped or isolated near the surface of the columns. A reticular network of the cytoplasm is often prominent. The spherical nuclei stain lightly except for a densely-staining nucleolus. There are from two to seven irregular layers of nuclei in the columns, some lying near the basal membrane.

The structure and arrangement of the *pars tuberalis* is more discernible in the large alligator than in the turtles. The condition of the individual animal may have a bearing upon this point. The two parts of the *pars tuberalis* lie in such proximity in the 150 cm. specimen, that they may be said to fuse, although they are distinctly separated in the smaller adults. The *pars tuberalis* is composed of irregular columns of cells separated by a large amount of vascular connective tissue. The columns are formed of clear, vacuolated, non-granular, polyhedral cells with a reticular network. The borders of the columns occasionally stain intensely. Cell walls are usually distinct. The nuclei—round, oval, or irregular in outline—do not stain deeply, except for a distinct membrane and for one, sometimes two or more, small densely-staining nucleoli.

The proximal parts of the lateral lobes, forming bands encircling the anterior lobe, are composed of columns. These are somewhat smaller, more irregular, possibly less vascular than those of the *pars tuberalis* and are separated by a thin connective tissue layer. The histologic structure is like that of the *pars tuberalis*, although there are tubules running through ten or more sections and ending blindly. The tubules are lined with columnar epithelium with basal nuclei. Cell membranes can be seen. Both the cytoplasm and nuclei stain more densely than in the surrounding cords and columns. A reticular sub-

stance is frequently found in the lumina. Such tubules, having the appearance of ducts, are never found in the pars tuberalis proper. A definite connective tissue sheath separates the anterior end of these bands from the anterior lobe, but it disappears caudally, the bands blending into the ventral part of the anterior lobe and with it becoming enclosed in a dense fibrous tissue.

Irregular cords, with a more or less prominent border of cytoplasm, form the main part of the anterior lobe. The cells are polyhedral and stain irregularly, some taking eosin readily while others in the same section of a cord are almost as clear as those derived from the lateral lobes. In the clear cells a reticular network is present. With iron haematoxylin and orange G. some cells have a bluish-black granulation, others are yellowish-brown and finely granular, while a third kind are clear, showing a yellowish-tinted reticulum (fig. 59). Near the border, particularly near the ventral side, the darker staining cells are less numerous. There are three or more rows of round nuclei, flattened or irregularly indented, in the center of the columns. They have a definite membrane, distinct but fine chromatin network, and one or several prominent nucleoli. In the clear, lightly-staining cells, the nuclei are near the center of the cell, while in the other cells the position is eccentric, frequently lying toward the center of the cord.

In the alligators received earlier in the fall no secretory masses were found in the pars intermedia, and the occasional, deeply-staining peripheral borders are less prominent. The cells of the pars tuberalis are small and irregular, and there are only a few clear cells in the anterior lobe. However, some cells of the anterior lobe stain bluish and others a deep pink with haematoxylin and eosin. In the large alligator, the many clear non-granular cells of the anterior lobe and the densely-staining cells of the pars intermedia and anterior lobe may be due to the activity of the animal before being killed. Cushing and Goetsch's ('15) study of the hypophysis of the opossum during hibernation suggests that the smaller alligators may have been in a similar state.

The free end of the cystic tubule, described in a 47.5 cm. alligator as lying in the ventral part of the anterior lobe (fig. 34), possibly representing the vestige of the stalk and closely resembling a similar tubule noted in an adult *Sceloporus*, is lined by one or two layers of cells. Some of the cells are large spheres with a reticulated cytoplasm and flattened basal nuclei. Others are small, with central oval nuclei and, as in *Sceloporus*, bear cilia on the inner surface. A reticular substance is found in the lumen. That part of the tubule imbedded in the anterior lobe is about twice the diameter of the free end, and is lined by a low cuboidal, non-ciliated epithelium. The tubule terminates blindly at either end. In the 150 cm. alligator a small cord occupies the same position as the tubule just described. Many cystic tubules present in the caudal end of the anterior lobe in most alligators contain a reticular substance which may take an acid or basic stain.

Discussion

In all the reptiles the cells of the *pars intermedia* are light-staining and non-granular, except in alligators where the peripheral cytoplasm of some cells lying next to the infundibulum stains more deeply with eosin. Sterzi ('04) described large and small cells in the *pars intermedia* and stated that the large cells retained iron haematoxylin. Investigators usually describe this part as faintly-staining. Tilney ('11) and Stendell ('14) considered the cells of this part basophilic.

The question as to whether or not there are two kinds of cells in the anterior lobe, has occasioned a great deal of discussion. Sterzi ('04) described three kinds of cells, some clear, others with many chromophilic granules, and a third kind with few. Tilney ('11) and Stendell ('13, '14) represent recent exponents of the two views concerning the cells present. Tilney believes there are two distinct types; acidophilic cells with nuclei occupying eccentric positions, found usually in the center of his '*pars distalis*;' and large peripheral basophilic cells with central nuclei. Stendell ('13) described acid or basic chromophilic cells and non-granular chromophobic cells. He discussed the opposing

views and believed the cells to be in different functional stages, of which the granular acidophilic cells represent the completed stage of secretory elaboration. Apparently Cushing ('12) is inclined toward the former view and Herring ('08 and '13) toward the latter. In the turtle and alligator material which I have studied, deeply basic-staining, granular cells, acidophilic granular cells and large clear cells are found. In the latter the nuclei are central in position. In snakes and lizards, cells taking varying amounts of eosin and others quite clear, are present. Unlike Herring's results, tubules containing colloid surrounded by clear cells were not found in turtles. Both granular and clear cells are always present. In alligators and turtles there are many of the deeply-staining cells, both acid and basic, in the center of the anterior lobe, while Tilney found the basic cells usually at the periphery. Although Haller ('96) described and figured a granulation toward the lumen or center of the cord, my material shows both proximal and distal granules, with possibly more at the basal end.

The author realizes with Herring ('08) that "It is extremely difficult to decide whether these appearances indicate distinct forms of cells or whether they are merely expressions of different functional stages of one and the same kind of cell." Since, in reptiles, some of the cells are densely-staining (acid or basic), often with basal nuclei, and others scarcely stainable, with central nuclei, it would seem that the latter may be cells which at the time contain no secretory granules. That the presence of two distinct kinds of cells in the anterior lobe can be explained by an ectodermal and an entodermal origin (Miller '16) seems doubtful. In the pars tuberalis and the part surrounding the anterior lobe, large clear polyhedral cells are present, and, in the latter part, occasional tubules lined by low, columnar, dense-staining cells occur. Staderini ('05) stated that the isolated masses derived from the lateral lobes in *Gongylus* are histologically like the remainder of the hypophysis, while Sterzi ('04) and Tilney ('11) considered the tongue-like process or pars tuberalis like the anterior lobe. In the cat, Herring ('08) distinguished, by histological characteristics, a tongue-like process

and a part covering the body of the posterior lobe. He described acini lined by clear cells and containing colloid in the former. More recently Tilney ('13) has given an excellent description of the pars tuberalis in mammals and birds. In the cat it is vascular and has an acinar arrangement of small, basophilic cells, sometimes granular, with either large or pycnotic nuclei. In the adult fowl, the cells grouped in masses are faintly or deeply-staining basophiles. In the pig, Miller ('16) described a cortical zone of chromophobic cells around the anterior lobe. There seems, then, to be a complete homology of these parts in the forms described.

In accordance with Tilney's conclusions from the cat and fowl, histologically there are three distinct parts of the adult epithelial hypophysis in turtles and alligators, but only two in snakes and some lizards (*Sceloporus*).

Lothringer ('86) stated that hypophyseal cysts, lined by a ciliated epithelium, are present in rabbits. Other investigators—Peremeschko ('67) and Thaon ('07) (according to Stendell)—have described a ciliated epithelium bordering the residual lumen of the hypophysis. Stendell is inclined to doubt the correctness of these observations in mammals. The presence of ciliated cells in the hypophysis of the higher vertebrates is difficult to explain.

Secretions

Tubules, containing a homogeneous substance or a reticular meshwork in their lumina are found in the anterior lobe of the hypophysis of turtles, occasionally in lizards, and in the anterior lobe proper, as well as in the band encircling it, in alligators. This is evidence of a secretion being emptied into these spaces. In turtles both the central and proximal ends of some of the cells are granular and darkly staining. In alligators usually only the peripheral part is granular.

In the peripheral zone of the pars intermedia of the large alligator, round homogeneously-staining masses are present. These may be as small as the nucleoli or larger than the nuclei, and are found in groups or as single masses. Such masses occa-

sionally occur in the peripheral zone of the infundibular wall but none were observed in its cavity. However, there are many cylindrical spaces, containing no stainable substance, in the infundibular wall. In one lizard numerous small round masses, varying in size and staining a deep blue (eosin-methylene blue) occurred in the cells of the pars intermedia. In some snakes darkly-staining round masses about the size of the nucleoli are present in many of the reddish-tinted cells of the anterior lobe. These masses are eccentric in position, often lying near the periphery of the cell.

Stendell ('13) described somewhat similar masses, which he suggested might be coagulated secretion, in the cells of the intermediate lobe of the elasmobranchs and amphibians. However, he found the masses vacuolated and staining yellow with picric acid. In reptiles they are not vacuolated and stain with eosin, iron haematoxylin and orange G. or with methylene blue.

Nerve supply

In turtles stained by Ranson's modification of Cajal's method there is no evidence of any nerve fibers entering the hypophysis from the infundibulum. In all of the specimens, except one, stained by various methods, a definite, uninterrupted, fibrous layer is found between the infundibular outgrowth and the pars intermedia. In the large alligator, however, there are many finger-like projections of the infundibular wall into the surrounding connective tissue and tubules of the pars intermedia. These are especially numerous in the region of the anterior end of the pars intermedia and come from all sides of the infundibular outgrowth. Many small projections pierce the connective tissue sheath, spreading fan-like, and apparently ending in the connective tissue. Other longer projections seem to be continuous with the epithelial columns; at least no connective tissue separates them. In these latter projections it is often difficult to distinguish between epithelial and nervous parts. Indeed, the nervous end appears as an epithelial cord in which the cells are markedly vacuolated and resemble the centers of some glandular

cords. In tracing such vacuolated cords one can always find a place where the connective tissue is interrupted and the cords are continuous with the nervous tissue. This tissue is reticular, like that of the infundibular wall, but it is questionable whether it can be considered as nerve fibers.

Various investigators have described nerve fibers coming from the infundibular outgrowth and entering the hypophysis. Sterzi ('09) believed such to be the case in elasmobranchs, and Stendell ('14) accepts this. Johnston ('01) was sure such was not the case in fish; Dendy ('13) found only a sympathetic supply in mammals; while Tilney ('13) states that many nerve fibers lie around and are imbedded in the pars tuberalis of the cat.

Connective tissue and blood vessels

In all reptiles the hypophysis is separated from the cranium by a connective tissue layer. This is extremely thin in snakes. Staderini called attention to the proximity of two muscle masses at the sides of the hypophysis in *Gongylus*. These are found also in *Lacerta*, as noted by Gisi ('07), and in *Sceloporus*. In turtles two lateral muscle masses are also present, but they are farther removed from the hypophysis. Pigment in the connective tissue surrounding the hypophysis is found only in lizards. Gisi found pigment in *Lacerta* and noted its absence in turtles, although described as being present by Stieda.

Layers of connective tissue surrounding the various parts of the hypophysis have been described. Although Tilney states that connective tissue is not present in the hypophysis of snakes, I find it in snakes as well as the other forms studied. In *Sceloporus* at least, the pars intermedia and anterior lobe are not completely separated by connective tissue. There is relatively more connective tissue in the pars tuberalis of turtles and alligators than in the remainder of the hypophysis.

The carotids border the sides of the hypophysis in all reptiles. Only in alligators and turtles is an anastomosing branch between them present. This is caudal to, or imbedded in, the caudal wall of the hypophysis and, in turtles, first appears in 5 mm.

embryos. An injected alligator shows no direct branches from the carotids to the hypophysis. However, the sinusoidal capillaries of the various parts are continuous, as is shown by various injected specimens. A large venous channel, caudo-dorsal to the hypophysis in lizards and snakes (fig. 41) apparently drains the organ. Many veins are found in the connective tissue surrounding the hypophysis in alligators.

HISTOGENESIS OF THE HYPOPHYSIS

The embryonic material used for this study was sectioned in paraffin, the sections being from 5 to 10 micra thick. Various stains were used and these are noted in the description, which is based mainly on turtle material, with references to other forms where necessary.

In very young turtles (2.6 mm.) and snake embryos (3.3 mm. *Vipera verus*) the hypophyseal evagination is composed of a single layer of low columnar cells with a distinct basement membrane. The cuticular border and cell membranes are distinct. The large spherical nuclei, basal in position, contain one large nucleolus, but very little other chromatin material, and have a definite nuclear wall.

The hypophysis in turtle embryos, 3 to 5 mm. in length, has two or three rows of oval nuclei containing one or two large round nucleoli and smaller chromatin masses. The latter are peripheral in position and are sometimes connected by chromatin strands with the eccentrically placed nucleoli. The cuticular border and cell membranes are less distinct than in younger embryos. On all sides, the pharyngeal roof is composed of one layer of columnar cells with oval nuclei, although farther from the hypophysis the cells are low columnar or cuboidal with spherical nuclei.

From three to five interlocking rows of centrally-placed nuclei are found in the wall of the hypophysis of 7 to 9 mm. turtle embryos (figs. 53 and 57). The nuclei are elongate and contain a large nucleolus and a dense chromatin network. Thin inner and outer cytoplasmic zones, staining darker than the

stalk or pharyngeal roof, are present. The pharyngeal roof is two-layered, having an inner columnar, and an outer flat epithelial layer which, in the stalk, is replaced by a columnar layer of cells.

The nuclei of the hypophyseal wall, three to five rows deep, are oval in a 14 mm. embryo (fig. 54). In the glandular outgrowths present at this stage, usually two, three, or even more rows of round nuclei are found arranged about the clear, central cytoplasm or occasional lumen. The nuclei about the original hypophyseal lumen are basal in position so that a narrow cytoplasmic zone borders the cavity. Cell walls and the cuticular border are indistinct, but the basal membrane is still prominent. Mitotic figures border the lumen in all the stages described. The one or two spherical nucleoli are relatively very large. They are present also in the nuclei of the mesenchyma, epithelium of the oral cavity, and the nerve cells. The stalk is lighter staining than the hypophyseal epithelium and shows signs of degeneration.

The entire hypophysis in a 28 mm. *Aromochelys* embryo is formed of solid cords of tubules with minute lumina. A very small residual lumen is present in the pars intermedia. The latter is formed of glandular cords, sometimes with enlarged ends, radiating in all directions, except toward the infundibulum (fig. 58). There are four or five rows of oval nuclei in these cords and in the roof adjacent to the infundibulum. Near the caudal end of the floor of the anterior lobe, a cystic tubule, the remnant of the stalk, occurs in some specimens. It contains a reticular substance and is lined by a single layer of columnar cells. The other tubules of this part have very little cytoplasm and three to five rows of nuclei. Sections of several cords of the anterior lobe and the thin lateral buds are shown in figure 55. In the cords the nuclei are almost round and some are very dark. Only occasionally does the cytoplasm of a cell show beginning differentiated staining. In the much lighter staining lateral buds (fig. 55) the nuclei are somewhat larger. All contain a prominent nucleolus. The attachment of the lateral buds to cords of the anterior lobe can be seen in some sections (fig. 8).

In very young adult *Chrysemys*, the cells of the anterior lobe are arranged in cords, three to five cells thick. The staining reactions are much less marked than in older adults. Cells staining homogeneously with eosin, others clear and vacuolated, and a third kind staining darkly with iron haematoxylin occur in the same tubule. The nuclei are oval, the cytoplasmic zone narrow. The nucleolus is distinct and the chromatin network dense. A thin layer of narrow cell strands forms the pars tuberalis. Here the cytoplasm is light-staining and the nuclei elongated. The laminae of the pars intermedia are four or five layers deep; the cells show no granules and the nuclei stain darker than those of the anterior lobe.

In 12 mm. *Tropidonotus* embryos there are about five rows of elongated nuclei in the hypophyseal wall. The surface of Rathke's pouch is regular, while many epithelial buds project from the surface of the anterior lobe. The basal membrane is indistinct. The nuclei of the distal ends of the lateral buds are spherical while those of Rathke's pouch and those lining the lumen of the anterior lobe are elongate. The entire anterior lobe of a 10.5 mm. *Eutaenia* is a mass of glandular cords and tubules. Many thickened regions in the wall of the ventral part of Rathke's pouch and the occasional extensions of the lumen into them indicates beginning glandular development from this part.

In a 10 cm. (total length) *Tropidonotus* the pars intermedia is a thick-walled, flattened vesicle (fig. 38) from which only very few glands are, as yet, outlined. In the inner layer of cells surrounding the lumen, the nuclei are oval, while in the outer three or four layers the nuclei are less crowded and are spherical. The glandular buds of the anterior lobe are long, sometimes dilated at the ends, where there may be a small lumen or cytoplasmic zone. The lumina are surrounded by a two layered epithelium containing round nuclei. One can hardly characterize the cells of the hypophysis in embryo alligators as lymphoid tissue, although Reese so regards them. Bruni has stated that a differentiation in staining reaction of the cells is present in a 44.7 mm. *Gongylus* embryo where the hypophysis is composed of

cords. In a 28 mm. *Aromochelys* embryo, differentiation is possibly just beginning.

From a study of my material it appears that Valenti has over-emphasized the distinction in staining reaction of ectoderm and entoderm in reptiles. The cells appear similar in shape and arrangement. Very early, the cells of the hypophyseal anlage are columnar and stain more darkly than either ectoderm or entoderm. It would appear difficult from histological characteristics to exclude the entoderm from taking part in the formation of the hypophysis.

Glands. As has been described by other investigators, in all of the specimens examined glandular outgrowths appear, first, from the wall of the anterior lobe. But this outgrowth is from all sides of the lobe, not from the anterior wall only, as Herring ('08) found in mammals. In very young turtle embryos one or two projections, caudo-dorsal to the stalk, are probably glandular outgrowths and may correspond to Woerdemann's ('14) 'solider Auswuchs' which this author thinks is of special importance. In alligators 22 mm. in length there are a great many evaginations from the cerebral side of the anterior lobe, though there is only one long, caudally-projecting outgrowth from the lower surface. In alligators many of these outgrowths show a distinct lumen. In other forms they are usually solid, with only slight extension of the main lumen into them. In snake embryos 10 cm. long, the glands occasionally show a lumen or a central cytoplasmic zone near their free ends. In alligators, at least, the glandular outgrowths seem to be evaginations from the hypophyseal wall, with lumina extending into them, and not solid cords formed by the breaking up of a very much thickened wall as Joris ('07) described in mammals. These evaginations may later become solid cords. However, since the lateral buds become solid very early, cords are probably formed by the breaking up of their walls or as outgrowths from them. The hollow pars intermedia of a 10 cm. snake shows no cords, while the solid pars intermedia of a 12 cm. *Eutaenia sirtalis* has several cord-like extensions.

Lumina. As noted by other investigators, the slit-like lumen disappears early from the lateral buds in all reptiles. A very small lumen is present in the pars intermedia of a 21 mm. turtle embryo, though two very young adults also show a lumen in the anterior lobe. A 12 cm. *Eutaenia sirtalis* has a small lumen in the anterior lobe (fig. 40) while in a 10 cm. *Tropidonotus* this is continuous with a flattened, slit-like lumen in the pars intermedia. In 12 cm. alligators there is a small lumen extending into the pars intermedia from the caudal part of the anterior lobe, while in a 37 mm. *Lacerta viridis* the lumen of the anterior lobe is connected with a larger one in the pars intermedia. It appears, therefore, that in turtles and lizards a lumen persists in the pars intermedia for a longer time than that in the anterior lobe. The contrary seems true for snakes and alligators, the lumen of the anterior lobe persisting longer than that of the pars intermedia. Gaupp ('93) found a lumen in the pars intermedia in a 60 mm. *Anguis* embryo, but it was not present in older specimens. Bruni ('13) described a lumen in the caudal part of the hypophysis in 44.7 mm. *Gongylus*. A persistent lumen has already been described in the pars intermedia of adult *Sceloporus*.

Although in young embryos mitotic figures are always central in position, there is no glandular ingrowth into the lumen as Herring ('08) found in the cat. It would appear that the lumen in reptiles is not closed off by inward glandular growth.

CONCLUSIONS

The epithelial portion of the hypophysis develops as a single anlage in turtles, lizards and snakes and probably in alligators.

In the development of the evaginations of the hypophysis Rathke's pouch appears first, then the two lateral buds, and finally the anterior bud.

The lateral buds in turtles give rise to the part termed by Tilney 'pars tuberalis,' and to a thin cortical zone around the middle of the anterior lobe; in alligators to the pars tuberalis and two bands encircling the anterior lobe; in lizards they appear

to persist as isolated masses or to disappear; while in snakes they completely disappear. The cortical zone or bands present in turtles and alligators have not been described in other vertebrates except in pig.

The tip of Rathke's pouch gives rise to the pars infundibularis (Tilney) or pars intermedia of the adult. The remainder of Rathke's pouch and the early anterior bud give rise to the adult anterior lobe, except for the thin cortex or band around it in turtles and alligators.

The three parts of the adult hypophysis are distinct ontogenetically and histologically.

The pars infundibularis or pars intermedia has a laminar arrangement of columnar clear-staining cells. The parts derived from the lateral buds are arranged in columns (or sometimes acini) of clear-staining polyhedral cells. The anterior lobe proper is formed of columns or acini, with clear-staining and darkly-staining cells which may be acidophilic or basophilic. In general, the pars intermedia and the parts derived from the lateral buds may be considered the chromophobic and the anterior lobe the chromophilic part.

ADDENDUM.

P. L. Schroeder, working in my laboratory, studied the hypophysis of ten newly-hatched *Chrysemys marginata*. In a reconstruction of one of these he shows a persisting hypophyseal stalk extending through the cortical band (derived from the lateral lobes) and the cranium almost to the epithelium of the roof of the mouth. In several specimens the anterior lobe is short, the lateral lobes covering its anterior end. Considerable variation exists in the length of the lateral lobes, especially the pars tuberalis. A small lumen is present near the caudal end of the anterior lobe of several specimens.

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PLATE 1

EXPLANATION OF FIGURES

52 Transverse section of the anterior lobe of the hypophysis of an adult turtle showing part of the cortical band and some tubules of the anterior lobe. $\times 500$ (W. U. C. 200).

53 Sagittal section of the roof of the anterior bud of a 7.2 mm. *Aromochelys* embryo. $\times 500$ (W. U. C. 77).

54 Sagittal section of the roof of the anterior lobe of a 14.5 mm. *Chrysemys* embryo, showing glandular outgrowths. $\times 500$ (W. U. C. 97).

55 Transverse section of the anterior lobe and lateral bud of a 28 mm. *Aromochelys* embryo. $\times 500$ (W. U. C. 99).

A, anterior lobe

B, brain wall

L, cortical zone derived from lateral buds

l, residual lumen

s, capillaries

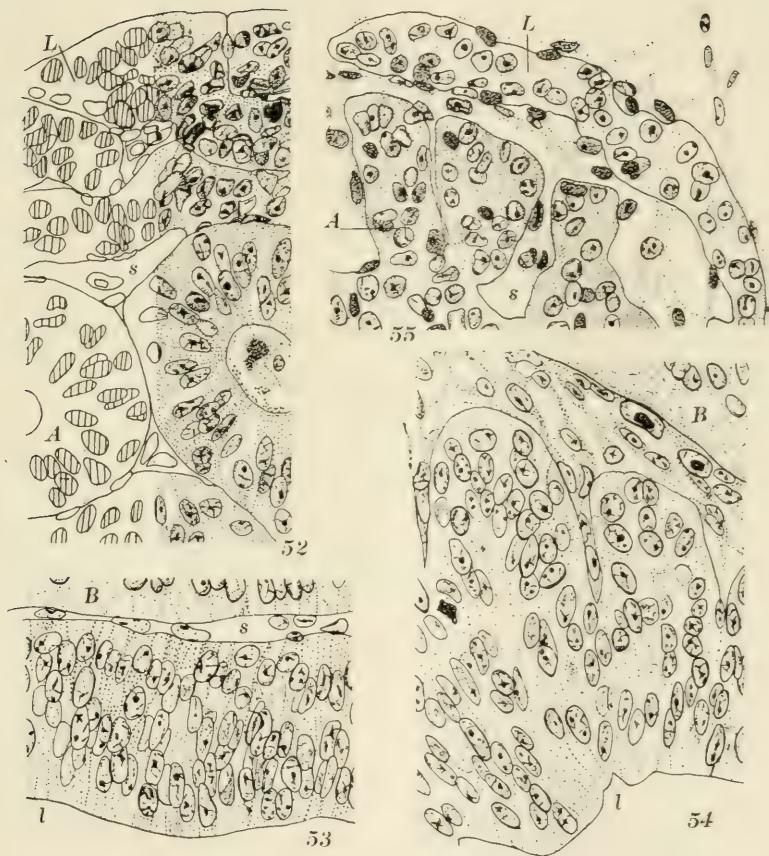


PLATE 2

EXPLANATION OF FIGURES

56 Transverse section of the pars intermedia and infundibulum of an adult turtle. $\times 500$ (W. U. C. 200).

57 Sagittal section of the tip of Rathke's pouch of a 9.2 mm. *Aromochelys* embryo. $\times 500$ (W. U. C. 91).

58 Transverse section of pars intermedia and infundibulum of a 28 mm. *Aromochelys* embryo. $\times 500$ (W. U. C. 99).

59 Transverse section of the anterior lobe of an adult alligator 150 cm. long. $\times 500$ (W. U. C. 210). Iron haematoxylin and orange G.

a, acidophilic cells

b, basophilic cells

c, clear cells

i, infundibulum

l, residual lumen

s, sinusoidal capillary



PLATE 3

EXPLANATION OF FIGURES

60 Left view of a wax reconstruction of the hypophysis of a turtle embryo, 4.6 mm. long. $\times 120$ (W. U. C. 79).

61 Same view of a 7.2 mm. embryo. $\times 150$ (W. U. C. 77).

62 Same view of a 17.0 mm. embryo. $\times 150$ (W. U. C. 42).

A, anterior bud or lobe

I, pars intermedia

L, lateral lobes

M, epithelium of mouth

R, Rathke's pouch

S, stalk

St, remnant of stalk.

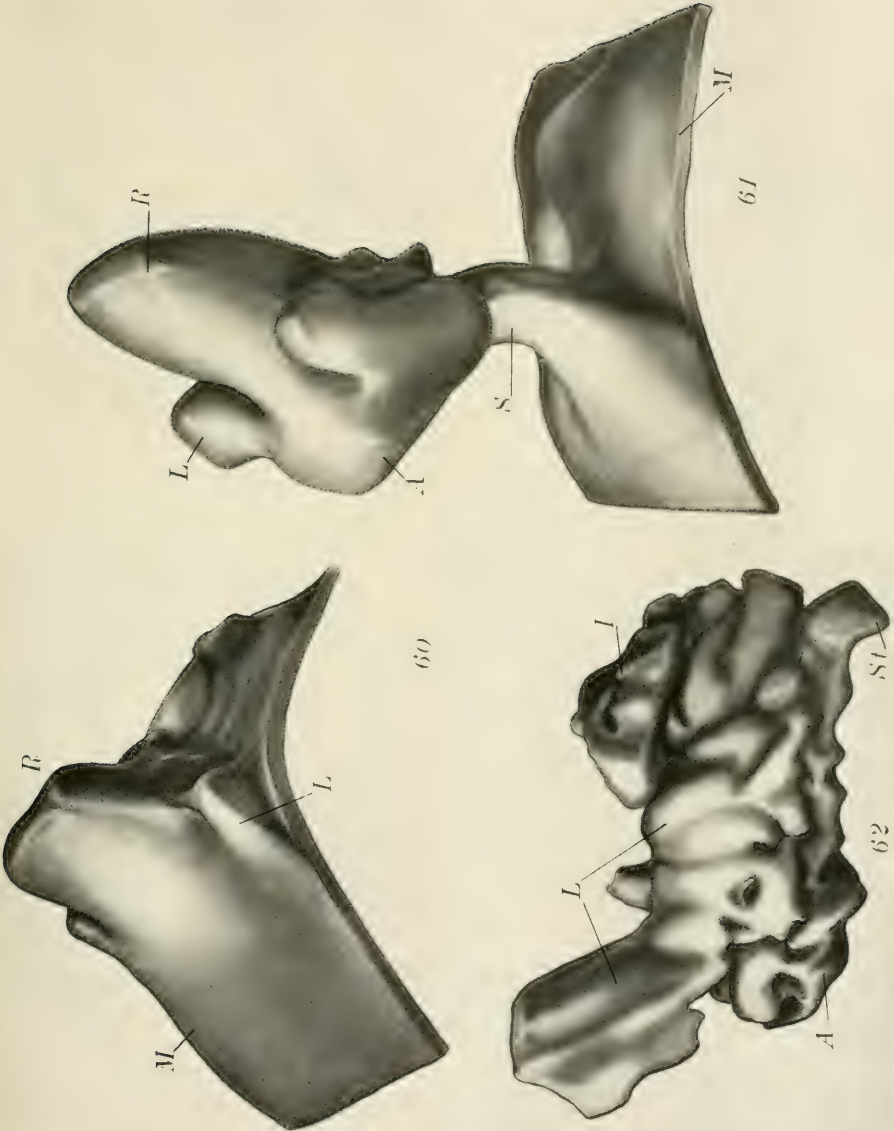


PLATE 4

EXPLANATION OF FIGURES

63 Anterior view of a reconstruction of the hypophysis of a 5 mm. *Lacerta agilis*. $\times 150$ (M. U. E. C. 559).

64 Anterior view of a reconstruction of the hypophysis of a 9 mm. *Lacerta agilis*. $\times 120$ (M. U. E. C. 576).

65 Left view of a wax reconstruction of the hypophysis of a 15 mm. *Lacerta agilis*. $\times 120$ (M. U. E. C., 562).

66 Anterior view of the hypophysis of a 5 mm. *Colubra* embryo. $\times 120$ (M. U. E. C. 567).

A, anterior bud

l, lateral buds

M, epithelium of mouth

R, Rathke's pouch

S, stalk.

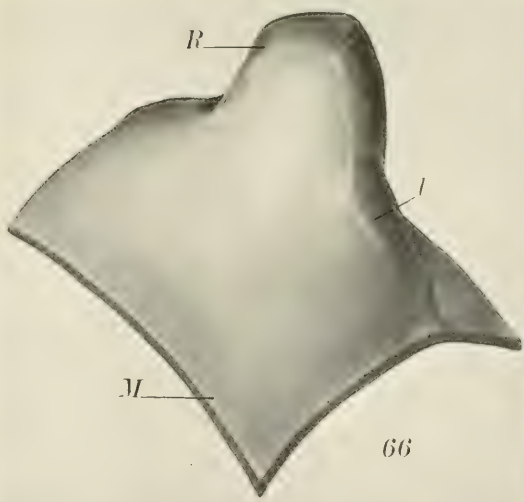
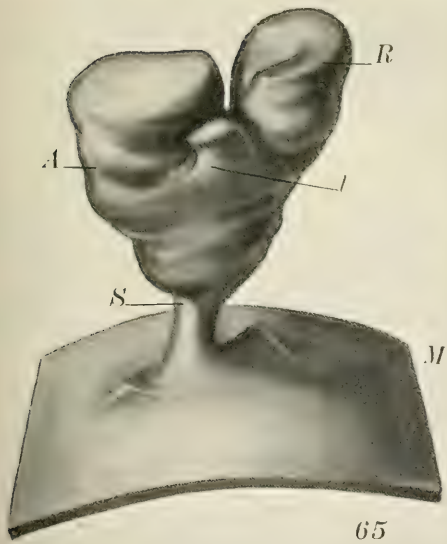
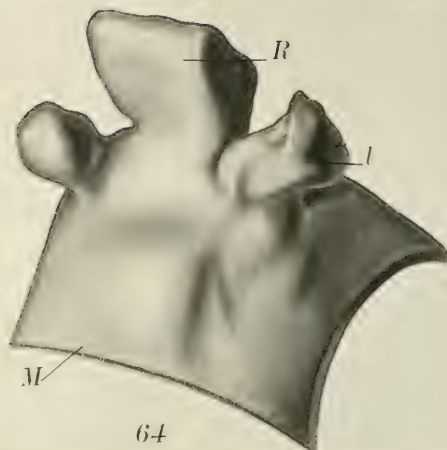
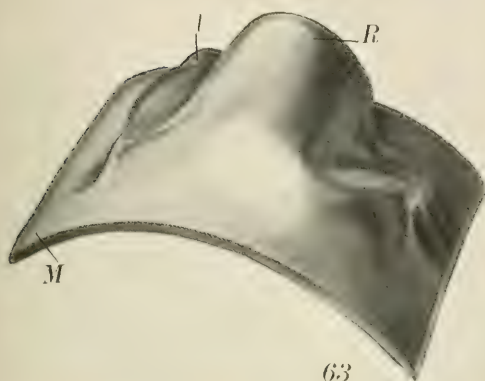


PLATE 5

EXPLANATION OF FIGURES

67 Right view of a reconstruction of the hypophysis of a 12 mm. *Colubra* (M. U. E. C. —). $\times 150$.

68 Left view of a reconstruction of the hypophysis of a 12 mm. alligator (R. C. 24). $\times 150$.

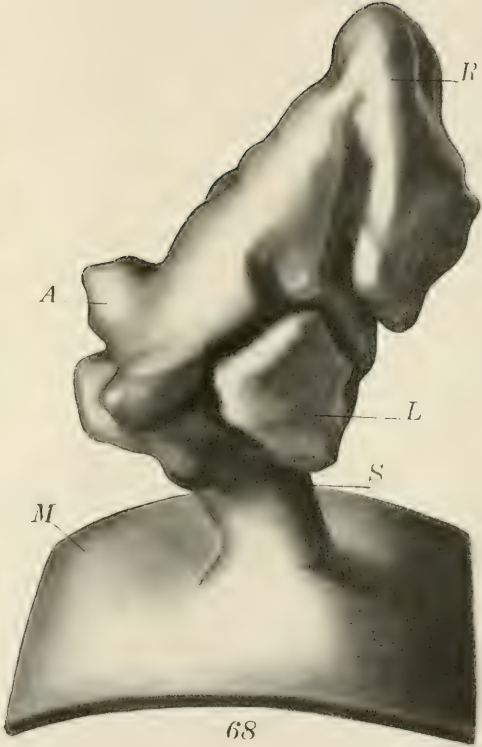
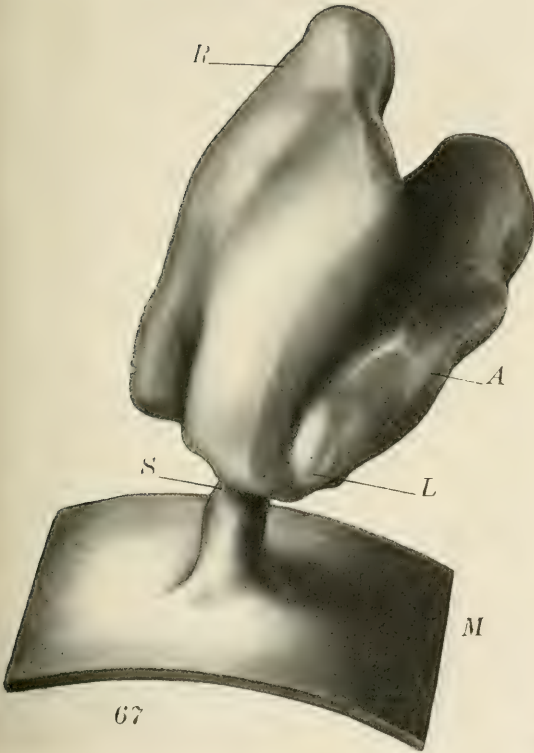
A, anterior bud

L, lateral lobes

M, roof of mouth

R, Rathke's pouch

S, stalk



A STUDY OF THE GERM CELLS OF *GRYLLOTALPA* *BOREALIS* AND *GRYLLOTALPA* *VULGARIS*

FERNANDUS PAYNE

*From the Zoological Laboratory of Indiana University*¹

FIVE TEXT FIGURES AND FOUR PLATES

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1. THE CHROMOSOMES IN MATURATION

A. Gryllotalpa borealis

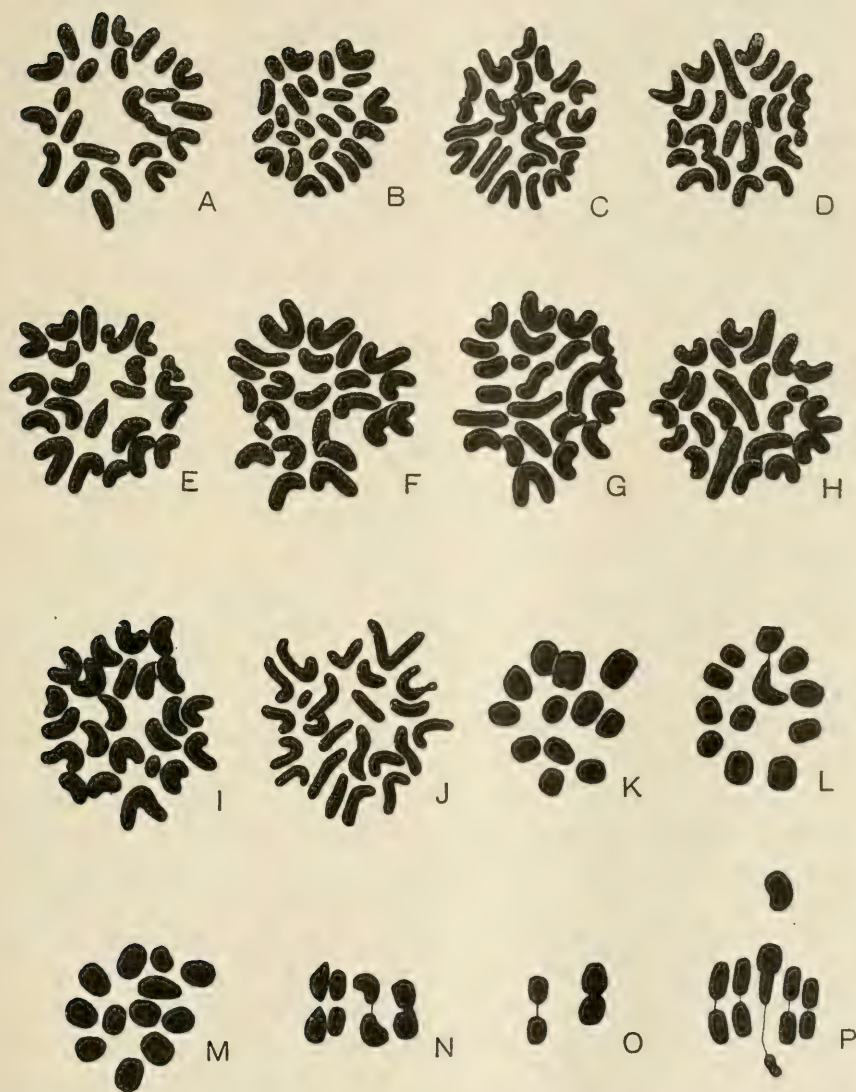
In Archiv für Zellforschung, 1912, I published a short account of the chromosomes of *Gryllotalpa borealis*. Since then I have extended my observations by an examination of a large amount of material, and have also been able to compare them with obser-

¹ Contribution No. 153.

vations on material collected at Naples, Italy, and Freiburg i. B., Germany.

Briefly stated, the results formerly published are as follows: There are 24 chromosomes in the oogonial cells and 23 in the spermatogonial. Of the 23 one is noticeably smaller than any of the others and smaller than any in the oogonial group. In the first spermatocyte division there are 12 chromosomes. If these chromosomes are studied in side view, 10 are found to be bivalent and dumb-bell shaped, with the two parts of the dumb-bell equal in size. Of the two others, one varies much in shape, due in part perhaps, to the position in which it happens to lie. It usually occurs to one side of the metaphase plate and may have reached one pole of the spindle before the other chromosomes divide. It does not divide in this division. The second of these two chromosomes is an unequal pair which divides unequally. The large part of the unequal pair and the single chromosome always pass in the first maturation division to the same pole of the spindle. This causes two classes of secondary spermatocytes, one with 11 chromosomes and one with 12. They differ not only in number, but also in that the 11-class gets the small end of the unequal pair, while the 12-class gets the larger. All of the chromosomes divide in the second spermatocyte division, hence two kinds of spermatozoa are produced, differing as did the secondary spermatocytes. As there are 24 chromosomes in the female and 23 in the male, the 12-class of spermatozoa is female producing, the 11-class male producing.

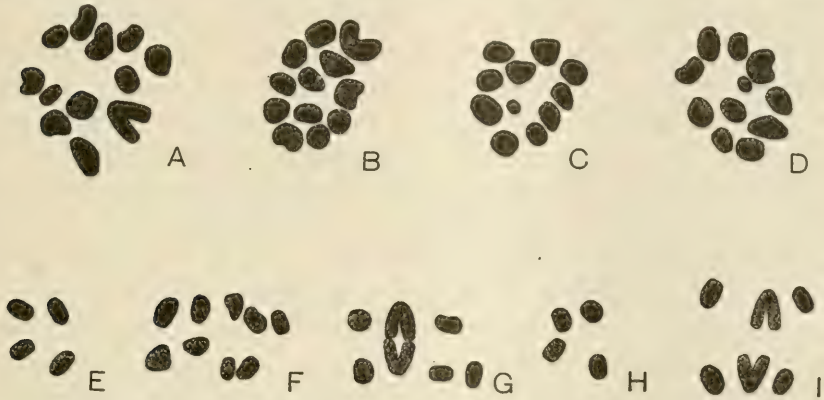
My present observations confirm, without exception, the earlier ones. At that time the count of the oogonial number (24) was made in only one individual. I now have counts in three individuals (fig. 1, A, B, C), and each one shows clearly 24 chromosomes. I should like to have made counts in more individuals, but the number of dividing cells in the ovary is very small and many ovaries show none. The young testes, however, show many, and here the number (23) has been counted in 12 individuals. In each case a small chromosome is present (fig. 1, D to J). No two of the spermatogonial groups are from the same male and hence are drawn from different slides. This



Text fig. 1 *Grylotalpa borealis*. A, B, C, oögonial divisions, 24 chromosomes; D, E, F, G, H, I, J, spermatogonial divisions, 23 chromosomes; K, L, M, first spermatocyte divisions, 12 chromosomes; N, O, P, side views of an early anaphase, first spermatocyte division, drawn at different levels in the same cell, to show all the chromosomes. (Note the position of the single unpaired chromosome and the precocious separation of the unequal pair).

accounts for the difference in the size of the chromosomes in the different figures. I lay emphasis on the spermatogonial and oogonial counts, as here is part of the evidence which demonstrates that the large end of the unequal pair and the single unpaired chromosome always go to the same pole in the first spermatocyte division. Polar views of the first maturation division show clearly 12 chromosomes (fig. 1, K, L, M). The single unpaired chromosome can usually be distinguished by its shape (fig. 1, K, L). The unequal pair can also be detected by changing the focus. In side views the two are always easily recognized, the single chromosome by its shape and position, the unequal pair by the inequality of its parts (plate 4, A to E). The single chromosome is seldom seen in the metaphase plate, but to one side of it. So far as my observations go, this is the same side on which we find the large end of the unequal pair. One hundred and fifty counts of metaphase plates show this to be the case. It may even have reached the end of the spindle while the other chromosomes are in metaphase. While there is no definite grouping of the chromosomes in this division, the unequal pair and the single chromosome lie somewhere near the center of the group. All the chromosomes, except the single unpaired one, divide in the first division. The unpaired member passes to one pole undivided and always goes to the same pole as the large end of the unequal pair. As previously stated, this distribution of the chromosomes causes two kinds of secondary spermatocytes. These secondary spermatocytes are alike in that each receives one-half of each of the ten equal bivalent chromosomes. They differ in that one receives the single unpaired chromosome and the large end of the unequal pair; the other receives the small end of the unequal pair. This also makes a difference in number, one receiving 12 chromosomes, the other 11. Figure 1, N, O, P, are three figures, side view, of an early anaphase plate taken at different levels. They show most of the chromosomes divided, the early division of the unequal pair, and that the single chromosome has reached the end of the spindle. Chromosome counts of the second maturation division confirm this method of distribution. Some have 12 chromo-

somes (fig. 2, A, B) and some 11 (fig. 2, C, D). In the 12-class a small chromosome is never present, while it is always present in the 11-class. All of the chromosomes divide in this division (fig. 2, E, F, G, H, four drawings taken at different levels in the same cell). Hence two classes of spermatozoa are produced, differing as do the secondary spermatocytes. One receives 10 autosomes, a single chromosome and the large end of the unequal pair; the other receives ten autosomes and the small end of the unequal pair. The counts in the oogonial and spermatogonial



Text fig. 2 *Gryllotalpa borealis*. A, B, C, D, second spermatocyte divisions. A and B the 12-chromosome class; C and D the 11-chromosome class; E, F, G, H, four figures of an early anaphase of a 12-chromosome, secondary spermatocyte, drawn at different levels in the same cell; I, anaphase of the second spermatocyte division, showing the further separation of the two parts of the single unpaired chromosome.

divisions also confirm this method of distribution, as there are 24 chromosomes in the female and 23 in the male. The male group always has the small chromosome, while the female group does not. This indicates that maturation in the egg is regular and that each mature egg receives 12 chromosomes. When the 12-chromosome class of spermatozoa fertilizes an egg, an individual with 24 chromosomes is produced, a female. When the 11-chromosome class meets the egg, an individual with 23 chromosomes is produced, a male. I wish to emphasize these facts, as some readers may be skeptical as to whether the chromosomes are always distributed as described. This skepticism may have

been increased by the recent work of Carothers and Robertson who have described an unequal pair among the autosomes, the distribution of which is at random.

a. *The chromatoid body.* Again some readers may suspect that the body which I have described as a single unpaired chromosome is a chromatoid body, similar to the one described by Wilson ('12) in *Pentatoma*. Wilson's principal reason for describing this body was to point out the danger of confusing it with an accessory chromosome. My reason for describing it here is to demonstrate that the single unpaired chromosome in *Gryllotalpa borealis* is not a chromatoid body. A chromatoid body is present, but it is not the structure which I described as a single unpaired chromosome. I have not attempted to trace the origin of the chromatoid body, but it appears as a deeply staining spherical structure in the early growth period (plate 1, B). It lies in the cytoplasm, near or in contact with the nuclear wall, and remains in this condition and appearance throughout the entire growth period. When the first spermatocyte chromosomes are in metaphase, it may lie in the metaphase plate (plate 4, A), at one end of the spindle (plate 4, E), or entirely outside the spindle (plate 4, B, C, D). At this time it usually stains intensely, as in the growth period, but occasionally it may stain and even look like a plasmosome (plate 4, D). When the cell divides it passes into one of the two secondary spermatocytes (plate 1, I). I have not followed it later than this stage, but it seems to disappear, rather than to pass, along with the cytoplasm, into the tail of the spermatozoon. A structure resembling this body is seen in the tail of the spermatozoon, but, as I shall show later, it is another structure. Both the chromatoid body and the single unpaired chromosome can be seen in plate 4, A to E. In this particular case there can be no danger of confusing the two structures.

B. Gryllotalpa vulgaris

a. *Introductory statement.* Since my preliminary paper went to press two short papers by Voïnov (12 and 14) have appeared, describing, in *Gryllotalpa vulgaris* Latr., an unequal pair

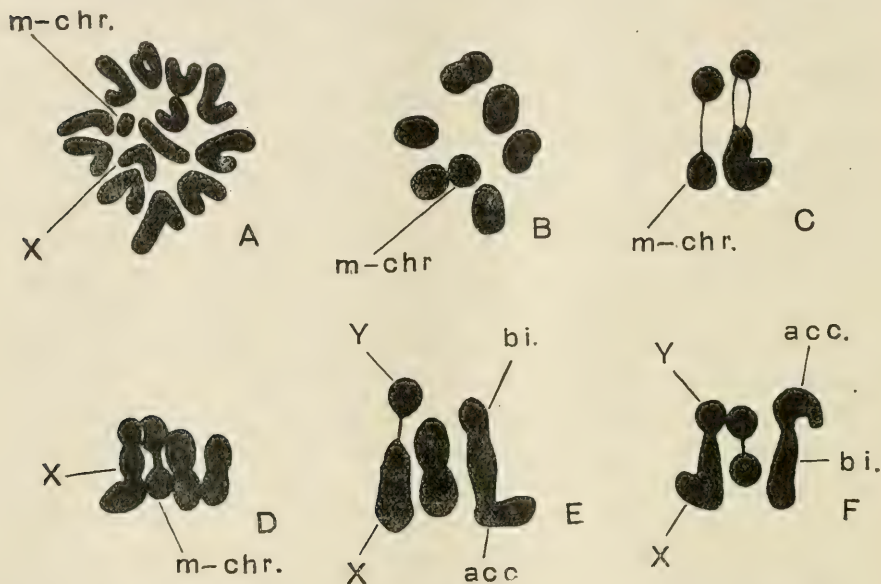
of chromosomes and an accessory chromosome, the distribution of which is a random one.

Fortunately, I also have some material of the European mole-cricket. I believe that *Gryllotalpa vulgaris* is the only species described in the whole of Europe. If there is only one species, I presume my material, some of which was collected at Naples and some at Freiburg, Germany, is also that of *Gryllotalpa vulgaris*. Mr. A. N. Caudell, of the Bureau of Entomology, Washington, D. C., has made a critical examination of my specimens and reports that he is unable to find any specific differences. The chromosomes, however, in the material collected at these places, are different. Whether this chromosomal difference constitutes a specific difference, matters but little. The principal point is that the material collected in these two places differs in at least this one respect. It seems that we here have a case similar to the one described by Wilson in *Thyanta custator* where he finds different chromosome numbers in material collected in New Jersey and in the South and West.

b. Observations of Voïnov. In *Gryllotalpa vulgaris*, Voïnov ('12) describes 14 chromosomes in the spermatogonia group (fig. 3, A). As one needs his figures to compare with my own, I have copied them. Figure 3, A, B, C, and D are from his first and figure 3, E and F from his second paper. One of the spermatogonial chromosomes, the small one, he labels 'm-chromosome' and another 'X-chromosome.' Just why he labels only one chromosome an m-chromosome, when he figures the m-chromosome in the first spermatocyte division as a bivalent, I do not understand. It seems to me there should be two m-chromosomes in the spermatogonial group. Further, I do not see how he is able to pick out the X-chromosome at this time. A side view of the X-chromosome in the first division (fig. 3, D) is also interesting. He figures and describes it as constricted into three parts. In this division he says one small part goes to one pole, the dyad to the other, while in the second division both parts divide. If he means to use the term 'X-chromosome' in the sense in which it has been used by American writers, I do not see how such a chromosome as he figures and describes

can be an X-chromosome. If he is using the term in some special sense, he fails to define it.

In his second paper ('14) Voïnov describes, among the seven primary spermatocyte chromosomes, one which he calls an L-chromosome (fig. 3, E, F). He believes this L-chromosome to be similar to that described by Sinety ('01) in *Leptinia*,² and



Text fig. 3 *Gryllotalpa vulgaris*. (After Voïnov). A, B, C, D, from his first paper, E and F from his second. A, spermatogonial division, 14 chromosomes; B, first spermatocyte division, seven chromosomes; C, the m and X-chromosomes, the X-chromosome dividing unequally; D, same as C, X has not divided; E and F, side views, first spermatocyte division showing XY pair and L-chromosome. In E the accessory is on the same side of the metaphase plate as X, in F it is on the same side as Y.

also to the hexad group observed by McClung ('05) in *Hesperotettix* and *Anabrus*. It resembles the letter L in shape, and Voïnov believes it to be made up of an accessory chromosome and a bivalent. He also figures an unequal XY pair. In one of his two figures he shows the accessory on the same side of the metaphase plate as the X-chromosome; in the other it is on the same side as the Y. He thus believes that the arrangement of

these chromosomes on the spindle is a matter of chance. This method of distribution causes four kinds of secondary spermatocytes. These according to Voïnov are as follows:

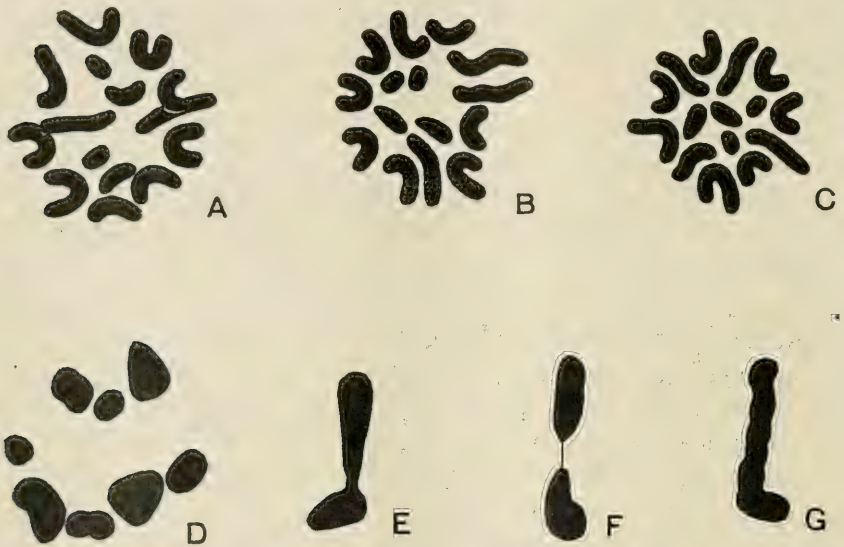
1. 4 dyads plus m-chromosome plus bivalent plus Y;
2. 4 dyads plus m-chromosome plus accessory plus X;
3. 4 dyads plus m-chromosome plus bivalent plus X;
4. 4 dyads plus m-chromosome plus accessory plus Y.

Voïnov does not discuss the relation of these four kinds of spermatocytes to sex production. Neither does he discuss whether the XY pair is related to sex production or whether it is an unequal pair of autosomes, as described by Carothers and Robertson.

c. A comparison of my observations made on material collected at Naples with those of Voïnov. Unfortunately I do not have enough good material to work out the chromosomal history in either the Freiburg or the Naples form. I wish, however, to compare the observations of Voïnov with those which I have made on the material collected at Naples. It seems probable that our observations have been made on the same form.

As mentioned before, Voïnov figures in the spermatogonial group one small chromosome which he calls an m-chromosome. In the first spermatocyte division he figures the m-chromosome as a bivalent. This would indicate that it is made up of two spermatogonial chromosomes, and I think such is the case. In my figures of the spermatogonial group (fig. 4, A, B, C) there are clearly 15 chromosomes and two of these are small. Hence it seems probable that Voïnov has overlooked one of these small chromosomes. My material showing the first spermatocyte division is inadequate and hence I cannot draw definite conclusions. It indicates, however, that there are eight chromosomes present instead of seven. This is what we would expect if there are 15 chromosomes in the spermatogonial cells and if the accessory is not linked to another pair. If it is linked as Voïnov describes, this would reduce the number to seven. I wish to point out at this place a possibility of error in Voïnov's interpretation. Certainly his figures are not convincing. The possibility of an error seems all the more probable when we compare his results

with those which I have obtained in *Gryllotalpa borealis*, where a study of a large amount of material leaves no doubt as to what actually takes place. In studying his figures the question arises whether the L-chromosome is really made up of an accessory and a bivalent. In the division of this chromosome Voïnov says the accessory passes to one pole and the bivalent to the other. This is not what we would expect if this chromosome is a bivalent plus an accessory. In fact we would expect the



Text fig. 4 *Gryllotalpa vulgaris* from Naples. A, B, C, spermatogonial divisions, 15 chromosomes; D, first spermatocyte division, eight chromosomes; E, F, G, an L-shaped chromosome.

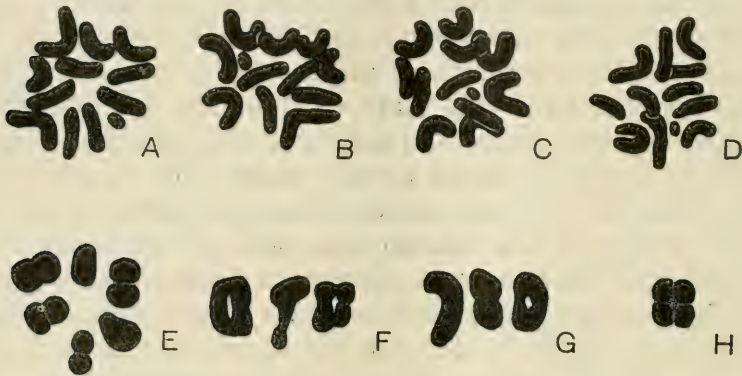
bivalent to divide equally, one-half of it passing with the accessory to one pole and the other half to the opposite pole. If Voïnov's interpretation be correct, we have here a bivalent (by bivalent I mean a first spermatocyte chromosome formed by the conjugation of two spermatogonial chromosomes) which divides in only one maturation division, an unheard of event, so far as I am aware, except in cases of irregularities. Further, in my observations on the Naples form, I find an L-shaped chromosome (fig. 4, E, F, G). Figure 4, F, shows it divided into two equal

parts. Voïnov does not show the L-chromosome in division, hence he gives no evidence of how it divides. In conclusion, it seems to me the points which I have mentioned make it very doubtful whether Voïnov has correctly interpreted his few observations and also whether his observations are adequate.

d. Observations of vom Rath and a comparison with my observations made on material collected at Freiburg. Vom Rath ('92) described the spermatogenesis of *Grylotalpa vulgaris*. He figured 12 chromosomes in the spermatogonial and six in the maturation divisions. Primarily because of the beautiful rings and tetrads his figures have been widely copied. After finding that the spermatogonial group in the material collected at Naples contained 15 chromosomes, I was anxious to obtain material from Freiburg, where vom Rath collected his, in order to see whether his observations were correct or whether the specimens collected in the two places actually differed in their chromosome numbers. Through the kindness of Professor Schleip a certain amount of this material was obtained. It was not sufficient to give the complete chromosomal history, but the spermatogonial groups were plentiful and very clear. They demonstrate without a doubt that the correct number is 12 (fig. 5, A, B, C, D). By comparing my figures with those of vom Rath, it will be seen that, while his number is correct, his drawings are very schematic. The same is true of his beautiful rings. To be sure rings are present in the prophase of the first division, but they by no means look like vom Rath's figures. Neither do the rings condense into such beautiful tetrads. Side views of the first spermatocyte chromosomes show that only one of the six approaches a typical tetrad (fig. 5, F, G). Figure 1, H shows this tetrad in a more typical condition. I mention this in the hope that text book writers in the future will eliminate such figures from their books. The few first division figures which are present in my material show six chromosomes (fig. 5, E). Figure 5, F and G, side views at different levels of the same spindle, show one of these bivalents unequal. As the spermatogonial group shows one small chromosome, it seems probable that this unequal bivalent is a pair of idiochromosomes.

C. Discussion of chromosomes

In the preliminary paper it was stated that the chromosome combination in *Gryllotalpa borealis* could be interpreted in one of three ways; first, as an unequal pair of idiochromosomes and a supernumerary; secondly, as a triad group similar to that of *Conorhinus*, *Fitchia* (Payne '09) or *Thyanta* (Wilson '10) with the exception that in *Gryllotalpa* the grouping occurs in the first division instead of the second, and that the two chromosomes which go to the female producing pole do not behave as a unit;



Text fig. 5 *Gryllotalpa vulgaris* from Freiburg. A, B, C, D, spermatogonial divisions, 12 chromosomes; E, first spermatocyte division, six chromosomes; F, G, two figures from the same cell, first spermatocyte division, side view; H, a fairly typical tetrad in the first spermatocyte division.

thirdly, as an unequal pair of idiochromosomes and an accessory chromosome. At that time I discussed the three possibilities, discarding the first as there was no evidence in support of it. The single unpaired chromosome is regular in its behavior and has a definite relation to sex production, always passing into the female-producing spermatozoon. Between the other two possibilities, I did not state my preference. I gave the facts in support of each and left the reader to choose for himself. At the present time, if I were to make a choice, I should select the second. Not because the evidence in itself favors it more than the third, but because this interpretation makes this combina-

tion no exception to the other sex chromosome combinations. That is, when viewed from a broad comparative standpoint, the group seems to be one similar to that in *Conorhinus*, *Fitchia*, and *Thyanta*, with the exception that here the grouping occurs in the first division instead of the second, and that the two chromosomes which go to the female-producing pole have lost the intimate association which they have in the other forms. If we consider *Grylotalpa* as an isolated case, the evidence is certainly in favor of the interpretation that we have here an unequal pair of idiochromosomes and an odd chromosome. The behavior of the single unpaired chromosome is typical of that of an accessory. It passes undivided in the first maturation division to one pole and divides in the second. The class of spermatozoa which receives it is the female-producing class, the other the male-producing. The behavior of the unequal pair is typical of the behavior of a pair of idiochromosomes. In the first division the two components pair to form an unequal bivalent, the two parts of which separate, the large end passing to one pole, the small end to the other. Both parts divide in the second. Of the two classes of spermatozoa thus produced, the one which receives the large end is female-producing, the one which receives the small end, male-producing. The only thing which indicates a relationship between the unequal pair and the single chromosome is the fact that the large end of the unequal pair and the single chromosome always pass to the same pole of the spindle in the first division. However, they are not intimately associated, as in *Conorhinus* and others, but pass separately and independently to the same pole. It is only from the comparative standpoint then that the second interpretation seems the more plausible.

In the preliminary paper, I also discussed briefly the arrangement and movement of the chromosomes on the spindle. It has been assumed that this arrangement is a matter of chance, an assumption made in order to explain Mendelian heredity. Until recently we had no direct evidence for or against such an assumption although it was generally accepted. I wish to state that I myself accept the theory and in the light of recent evidence

believe it comes very near being a fact. I make this statement because Carothers ('13) who describes in three species of grasshoppers an unequal pair of chromosomes which is arranged on the spindle according to the law of chance, states that I argue that there is no haphazard arrangement of the chromosomes. Based upon the fact that in *Gryllotalpa* we find two chromosomes, apparently independent of each other, moving to the same pole, I suggested the possibility that there may be law and order in the arrangement and movement of all the chromosomes, although the microscope does not reveal it to us. The large end of the unequal pair and the single chromosome are brought into the male by the egg. In maturation they always pass into the female-producing spermatozoon. They never enter the male line. This being the case I further suggested the possibility that all the chromosomes brought into the male by the egg may pass into the female-producing spermatozoon and those brought in by the sperm into the male-producing spermatozoon. I believed the facts in *Gryllotalpa* warranted the suggestion and I still believe so, although suggesting a possibility is not arguing in favor of it. While it is well to bring as much evidence as possible to the chance arrangement of the chromosomes and to refute any unjust evidence against it, yet, it seems to me, we must also keep a sharp lookout for other possibilities. It was from this standpoint that I made the suggestions.

If the chromosomes should move as suggested, I stated that one of the difficulties to be met would be the transmission of characters from father to daughter and mother to son. This expression, "father to daughter and mother to son" was perhaps not well chosen, and perhaps Carothers's criticism of it was just. What I meant was the transmission of characters from the male to the female line and vice versa. In order to explain such crossing I said we might assume the chromosomes to be made of units and that we might have an interchange of such units at synapsis. The latter part of this suggestion was not new. It is a part of the chiasma-type theory of Jansen and Morgan, and with the abundant evidence from the experiments of Morgan and his students, it seems that even the most conservative must

admit crossing over. To Carothers the chromosome is a unit and she believes all the facts of Mendelian heredity can be explained without assuming an interchange of factors at synapsis. She does not discuss experimental results which demonstrate crossing over. Carothers, however, does not meet smooth sailing everywhere. She has not examined the female cells, but of course has to assume that there is an unequal pair of chromosomes present and that, in maturation, either the small or the large end of the unequal pair may remain in the egg. As all the males have the unequal pair, she has to further assume that a spermatozoon with the large chromosome can fertilize only an egg with the small chromosome and vice versa. So far as I know, we have no direct evidence of selective fertilization, and I am not quite willing to admit it without such evidence. Further, Robertson ('15), who has described a similar unequal pair of chromosomes in several species of grasshoppers, does not find the unequal pair in all males, and he thinks Carothers may find this to be the case upon further study. He is not forced to assume selective fertilization.²

2. MITOCHONDRIA

My material was prepared for a study of the chromosomes. As that fixed in Fleming and stained with iron haematoxylin showed the mitochondria with particular clearness, it seemed worth while to describe them, especially so since there is still need for work in this field. Despite the fact that a large amount of work has been done, particularly by Europeans, conflicting views exist as to the origin, function, and fate of these bodies.

A. In the male sex cells

The spermatogonial cells are arranged in the form of a rosette (plate 1, A). Due to this arrangement, the inner ends of the

² In regard to sex inheritance I have nothing new to add, but I wish to correct a statement in my former paper ('12). I stated that Wilson had practically abandoned the quantitative theory of sex production. Wilson informs me that such is not the case and I wish to express my regrets for misquoting him. The error was due to misinterpreting a paragraph in his paper of 1911.

cells are narrower than the outer. At this inner end lies the mass of mitochondrial granules. In the early growth period these granules collect into a more or less spherical mass (plate 1, B). At this time several other bodies may be visible in the cytoplasm (plate 1, B). The one which stains intensely black is the chromatoid body. Its behavior has been described under another heading. As to the origin, fate or function of the other two bodies which look like plasmosomes, I have no data. When the mitochondria at this stage are sufficiently destained, a plasmosome-like body can be clearly seen in the middle of them. What this central body is, I am not prepared to say. Some observers might call it an idiozome, but I am not inclined to do so, as there is no indication of such a structure persisting immediately after the spermatogonial division.

Following this stage the granular appearance of the mitochondrial mass changes to that of a mass of threads (plate 1, D). This change seems to be due to the rearrangement rather than to the fusion of the granules. The mass remains in this condition throughout the rest of the growth period. Lewis and Lewis ('15), in their recent observations of mitochondria in living cells, maintain that a mitochondrion is an active moving body, constantly changing its shape and position. Certainly such cannot be the case here. At the metaphase of the first spermatocyte division the threads take a position on the outside of the spindle, extending about half way round it (plate 1, E and F, side views and plate 1, G and H, polar views of the metaphase plate). In side views, the threads appear as rows of granules, and in polar views the cut threads appear as single granules. After the chromosomes have reached the ends of the spindle, the mitochondrial threads divide approximately in the middle (plate 1, I). One mass of the divided threads moves along the spindle toward one end of it and half toward the other end. Plate 1, J, shows the mitochondria after they have reached the ends of the spindle. In this figure they appear granular again, but this I believe is due to the fact that the stain is almost completely extracted. At any rate the threads are present in the second maturation division. Their appearance

and arrangement here is very much as in the first maturation division (plate 2, A, anaphase second maturation division). Again the chromosomes reach the end of the spindle before the mitochondrial threads divide. Plate 2, B, is a late telophase of the second division. Here the mitochondria have reached the ends of the spindle and lie near the reconstructed nucleus. It will be noticed that there is no indication of the remains of a sphere at either end of the spindle. In fact, I believe in this case, at any rate, that the spindle and spheres completely disappear. Plate 2, C, is a spermatid shortly after the second division, and there are no indications of any such remains. I shall discuss this fact in connection with the formation of the acrosome. The spermatid now transforms into a spermatozoon, and it remains to follow the mitochondria in this transformation.

Plate 2, D and E, show an early stage, but here the mitochondrial mass remains very much the same as at the end of the second division. Two dark bodies, however, are present at this time. One of them is concerned in the formation of the acrosome, but they will be discussed in another place. The first change in the mitochondria is shown in plate 2, F. The granular appearance of the threads is entirely lost and they become arranged into a fantastic design which looks almost too diagrammatic to be true. The body which forms the acrosome has moved toward the opposite pole of the nucleus, or at least, the relative position of this body and the mitochondria has changed. In plate 2, G, the threads seem to form a series of concentric circles, with another thread running through the middle. This is the first indication of the separation into two halves. The axis cylinder has made its appearance at this time and is entirely separate from the mitochondria. Its point of origin is a small granule in contact with the nuclear wall. Whether this granule is a centrosome, I cannot say. The two halves of the mitochondrial mass now begin to elongate (plate 2, H). The inner circles lose their identity more or less as transformation proceeds. The axis cylinder, which has grown in length, now lies along the median line which separates the two halves of mitochondria (plate 2, I). The single granule which was the point

of origin of the axis cylinder is now double, and the two parts are larger and unequal in size (plate 2, J, K). The mitochondrial mass still continues to elongate. Viewed from the side, the stage shown in plate 3, A, appears as in plate 3, B. At this time the mitochondria in cross section appear as two cylinders (plate 3, G) between which lies the axis cylinder. What force brings these two structures into this intimate relation, I do not know. In plate 3, A, the mitochondrial mass is seen to be larger near the nucleus. This condition changes, either by a constriction of the mass at this point or by its backward movement, after which the mitochondria appear as rows of granules along the axis cylinder (plate 3, E, F).

The nuclear material has also been changing. From a loose network (plate 3, A), the chromatin has condensed until all traces of a network has been lost (plate 3, F). It will be noticed in this figure that the chromatin is denser at the periphery of the nucleus. This statement holds good for all later stages of condensation until the mature spermatozoon is reached. Following the stage shown in plate 3, F, the mitochondria lose their identity. Even the axis cylinder can no longer be seen. The tail appears to be made up of a homogeneous substance (plate 3, M to Q). Whether this disappearance of the mitochondria means a complete change or simply a condensation into a homogeneous mass, very much as the chromatin condenses in the transformation of the nucleus, I am unable to say.

B. In the female sex cells

The mitochondria in the male cells have been traced from the spermatogonia through the various stages of maturation into the tail of the spermatozoon. The question of their origin was thus left undetermined. This fact led me to examine the female sex cells in the material already at hand. In the oogonia at one side of the nucleus, there is a mass of granules similar in appearance to those in the spermatogonia (plate 4, F). These granules are, I believe, the mitochondria. As the cells increase in size during the growth period, the mitochondria move out

into the cytoplasm and also around the nucleus (plate 4, G). At this time there is a sharp line of demarcation between the mitochondria and the peripheral cytoplasm. As growth continues, the mitochondria move further out into the cytoplasm and the line of demarcation becomes less distinct (plate 4, H). Finally, at a later stage, before the appearance of the yolk in the developing egg, the mitochondria appear as minute granules equally distributed throughout the cytoplasm (plate 4, I). My drawings have been made from the ovaries of *Gryllotalpa vulgaris* (Naples) as here the mitochondria are much more sharply differentiated. Their behavior is the same in *Gryllotalpa borealis*.

C. Discussion of mitochondria

The principal questions which arise in connection with a study of mitochondria are: first, their origin; second, their continuity or discontinuity; third, their fate; and fourth, their zoological and physiological significance. Many suggestions have been made in answer to these four questions. It is not my intention to discuss the literature in detail, but merely to point out the principal differences of opinion and then give my own tentative conclusions, based on my observations in *Gryllotalpa*. For those who wish a detailed and critical review of the literature in this field, I refer them to that of Duesberg of 1911.

In a study of the spermatogenesis of the mouse, Benda ('97) observed some granules in the cytoplasm of the sex cells. He followed these granules through the different stages of maturation and into the spiral filament of the middle piece. While earlier workers had seen these granules, Benda was the first to describe them in detail and to ascribe to them any particular significance. From this early observation and later ones Benda concluded that these granules are specific cell organs of cytoplasmic origin; that they are present in all cells; that they are carried into the egg by the spermatozoon and hence play a part in heredity. To these granules Benda gave the name 'mitochondria.' While other names have been proposed and used, some perhaps even more appropriate (chondrioconten, plasto-

somen, chondriosomen, etc.), I have used the original term proposed by Benda.

Benda's conclusions have been supported, in the main, by the researches of Meves, Duesberg and numerous other workers. These workers have also added that the mitochondria are genetically continuous and that they differentiate during embryonic development into myofibrils, neurofibrils and various other filar structures. Opposed to these views are those of Goldschmidt, Popoff, Buchner, Wassilieff, Jordan and others, who maintain that the mitochondria are only temporary structures appearing in the cytoplasm, and that they are of nuclear origin. They homologise these bodies with the chromidia of Hertwig. Many differences in respect to behavior and other details have also been described by these two schools. Goldschmidt later ('09) admits that in embryonic cells mitochondria may differentiate into fibrils.

Schaxel, in a series of papers ('09, '10, '11 and '12), describes two types of granules in the cytoplasm, one of nuclear origin, the chromidia, and the other of cytoplasmic origin, the mitochondria. The chromidia, according to this author, are used up in differentiation, while the mitochondria are continuous cell elements. These observations might lead us to believe that different workers have seen different things and described them as the same thing.

Beckwith ('14) has given Schaxel's conclusions a thorough test in a study of the plasma-structure in the egg of *Hydractinia echinata*. She has employed a wide variety of fixing reagents and stains. Besides the yolk-spheres and oily bodies, she finds two types of granules, one which she calls pseudochromatin granules (perhaps the chromidia of Schaxel) and the other mitochondria. The pseudochromatin granules appear early in the growth period and transform into yolk. They arise in the cytoplasm and are not of nuclear origin. The mitochondria do not appear until the yolk is well formed. When they make their first appearance they are scattered throughout the cytoplasm and hence, as the author believes, cannot be of direct nuclear origin. By centrifuging the mature eggs she can cause the mito-

chondria to be thrown to one pole. In some cases the first cleavage plane cuts the egg so as to leave all the mitochondria in one of the first two blastomeres and none in the other. She found that each of these blastomeres, when separated, developed into a normal larva. She concludes that the mitochondria are not a vital part of the protoplasm, but only highly differentiated products.

Before discussing my own results I wish to describe briefly two observations which have been made on *Gryllotalpa vulgaris*. Buchner ('09) figures the mitochondria in the spermatogonial cells as very similar to those in *Gryllotalpa borealis* (plate I, A). The only chromatin he finds in the nucleus at this time is in two nucleoli, which are partly chromatic and partly achromatic. The rest he believes to have passed through the nuclear wall into the cytoplasm to form the mitochondria. According to Buchner the mitochondria disappear before the next division. Duesberg ('10), in the same form, finds the mitochondria very much as Buchner figures them but finds no proof that they are of nuclear origin. He also shows them to be present in mitosis. Here is a case where two observers working on the same material reach opposite conclusions. I can see how two observers working on the same material might differ as to interpretation, but I see no reason why they should differ so much in their observations. In this case one or the other has certainly made an error.

From my own observations I cannot speak as to the origin of mitochondria. They are present in large numbers in the spermatogonial cells. Judging from their mass here and in the growth period, it would seem that they increase, but, again, I have no direct information as to how this is accomplished. As I find within the mitochondrial mass in the early growth period, a spherical body which might be interpreted as a sphere, I wish to mention Vedovsky's viewpoint as to the origin of mitochondria. I have not seen his original paper, but Duesberg ('11) quotes him as stating that the mitochondria are simple products of the protoplasm, arising through the regressive transformation of the centropoplasm. Perhaps this central body in *Gryllotalpa*

is a centrosphere, and perhaps the mitochondria multiply at its expense, but I have no evidence that such is the case. In fact, I am not inclined to the belief that the centropasm or sphere ever gives rise to mitochondria. They have been figured as lying around the sphere, but this is no proof that they arise from it. In *Gryllotalpa* there is no indication that a sphere or the spindle and astral fibers persist after cell division.

Certainly there is no indication that the mitochondria arise from the nucleus. They persist throughout the entire growth period. In the first part they are granular, but later become arranged in elongated filaments. The filaments remain in a mass, however, until the prophase of the first spermatocyte division. In metaphase they are found on the outside of the spindle, with their long axis in the direction of the long axis of the spindle (see figures). They remain in this position until the chromosomes have divided and have moved to the ends of the spindle. The mitochondrial filaments then break in or near the middle, part moving to one pole and part to the other. In the second division they take a similar position and divide in the same way. I have no evidence as to the mechanism of this division. Montgomery ('11) states that the elongated threads do not divide autonomously in *Euschistus*, but are broken by the constriction of the cell body. This is not the case in *Gryllotalpa*, as the division takes place before the constriction of the cell has progressed far enough. Further, this constriction would not explain their movement along the spindle. As a result of the division of the mitochondrial filaments, each spermatid receives a mass of mitochondria and, as is characteristic of the insects, the mass takes part in the formation of the tail of the spermatozoon. While the identity of the mitochondria as granules or threads is lost in the last stages of the development of the spermatozoon, the earlier stages in this development indicate that they form a sheath for the axial filament. In the fully formed spermatozoon the tail appears as a homogeneous structure. The axis cylinder is not even visible.

In the female sex cells the mitochondria are present in the earliest stages observed (oogonia) and migrate out into the cyto-

plasm during the growth period. I have not studied the fertilization stages and hence cannot say whether mitochondria are carried into the egg by the spermatozoon. What few observations I have made, then, are not conclusive as to the origin or the genetic continuity of these structures. They indicate, however, that they are genetically continuous. At any rate, in the male, they do not arise in the growth period, but are continuous from the spermatogonial stage, through the growth period, the maturation divisions and the transformation of the spermatid. In the female they are present in the oogonia and continuous during the early growth period or as far as I have followed them. While I am willing to admit that the mitochondria may be continuous structures, I am not inclined to ascribe to them any value in heredity, at least in the present state of our knowledge.

3. THE ACROSOME

Late telophases of the second spermatocyte division show the reconstructed nucleus and the mitochondrial mass lying near it (plate 2, B). In this figure most of the spindle still remains, but it has partly disappeared at one end, where there is no indication of the remains of the aster to form an idiozome or Nebenkörper. In fact, in later stages, the spindle and asters disappear completely. Plate 2, C, shows there is nothing in the cytoplasm of the young spermatid except the mitochondria. I emphasize this fact, as the acrosome has been described as arising from the Nebenkern. The next stage following that shown in plate 2, C, is shown in plate 2, D and E. It will be noted that there are two additional bodies present at this time; one elongated, curved, and in contact with the nuclear wall, the other a small spherical body. Both stain very intensely with iron haematoxylin. Many cysts showing thousands of cells in this stage have been found. I have no knowledge of the origin of these bodies, although a large amount of material has been carefully examined. It would seem that they arise *de novo* in the cytoplasm as they are not present in the young spermatid. The spherical body cannot be the chromatoid body, as this structure is present in all the spermatids at this stage of transformation. Later it passes into

the cytoplasm of the tail and is thrown off. The elongated body remains to form the acrosome. It migrates to the side of the nucleus opposite the mitochondrial mass (plate 2, F to K) and condenses into a cone shaped cap (plate 3).

4. THE MIDDLE-PIECE

In *Gryllotalpa* the middle-piece arises from the same body from which the axial filament grows (plate 2, G). As the middle-piece has been described as arising from the centrosome in practically all cases, the presumption is that this body is a centrosome. It is only a presumption, however, as I have not been able to trace the body back to the centrosome of the second spermatocyte division. Whatever this small body may be, it undergoes considerable change in its transformation into the middle-piece. It first divides, or in some manner gives rise to a double structure, the two parts of which are at first approximately equal in size (plate 2, J). This equality changes with the enlargement of the basal granule (plate 2, K and plate 3, A, B, C, D). The double structure next changes to a more or less flattened single body (plate 3, E and F), which again soon changes to a double structure (plate 3, K). This double structure, when viewed from below, appears as two half moons (plate 3, I). At this time the axial filament can no longer be seen. The last stage in which the middle-piece is visible is shown in plate 3, O. The two parts here are again unequal. In the later stages of the development of the spermatozoon there are no traces of the middle-piece.

5. DISCUSSION OF ACROSOME AND MIDDLE-PIECE

In a study of the transformation of the spermatid into the spermatozoon I have been impressed with the small amount of cytoplasm which goes into the spermatozoon. Masses of it seem to be thrown off along the tail (plate 3, J). In the mature spermatozoon, certainly, there can be no more than a thin film surrounding the nucleus and tail. In fact, no cytoplasm can be seen around the nucleus. However, the acrosome, middle-piece and tail are extranuclear structures, and hence of cytoplasmic

origin. Just what part they play in fertilization is not clear in every case. It has been thought that the acrosome is nothing more than a cap of passive metaplasmic material which may aid the spermatozoon to penetrate the egg membranes. This is by no means an established fact. It disappears after the entrance of the spermatozoon, or, at any rate, we can no longer trace it. However, it does not necessarily follow that because it becomes invisible, that it has disappeared without playing any part in fertilization. In the last twenty years hundreds of papers have appeared on mitochondria. These bodies lie in the cytoplasm and in many cases, at least, seem to arise *de novo* and to disappear very much as the acrosome does. Why are we giving so much attention to one and none to the other? The same is true with the middle-piece. We are content to let it give rise to the centrosome. My point is that in our discussion of mitochondria and chromosomes we have almost forgotten that there are other structures in the cell. Some twenty-five or more years ago the acrosome was relegated to the scrap heap of functionless structures and there it still lies. Perhaps it should remain there, but recent studies, it seems to me, have demonstrated that we cannot scrutinize such structures too closely. Even those who have held and still hold that the chromosomes are the actual carriers of the heredity factors, are coming more and more to believe that these factors are only one of a series which determine the character in the adult. Likewise it may be that the middle-piece does more than merely carry the centrosome or incite the formation of one. Sometimes the tail enters the egg and sometimes it is left outside. What part, if any, it plays when it enters the egg is not clear. Meves and Duesberg, however, maintain that, in some cases, the tail enters the egg, carrying with it the mitochondria and thus plays an important rôle in fertilization.

6. CHROMOSOME NUMBERS AND SPECIES

From his studies on the Orthoptera, McClung has maintained that all the species of a closely related group should have the same number of chromosomes. While this has been true of his

own observations, I cannot see how he can continue to maintain this to be a fact applicable in all cases, especially so if he believes the observations of others.

Wilson has collected *Thyanta custator* in New Jersey and in the South and West. In the material from New Jersey there are 16 chromosomes in both sexes and in that from the South and West there are 27 chromosomes in the male and 28 in the female. Mr. Van Duzee and Mr. Barber have studied the specimens from the two localities carefully and fail to find any constant specific differences. Among the Reduviidae, I have studied five species of the same genus (*Sinea*) and find the number varies from 28 to 30 in the males and 30 to 34 in the females. In the family itself, so far as I have examined, the number varies from 22 to 34.

The attempt to make his hypothesis of general application has led McClung to make some erroneous statements. From his last paper ('14) I quote as follows:

Gryllotalpa has been studied by Buchner, Payne, vom Rath, and Voinov. Vom Rath ('91) reported at length on the spermatogonia of this animal and his figures were widely copied, but it is very evident that he was fundamentally in error regarding the most essential part of his work. He announces 12 as the diploid number but the internal evidence and the results of other investigators show that this is really the haploid condition.

What this internal evidence is he does not mention. Again in the same paper, he says,

vom Rath ('91, '92, '95) has reported the process of maturation in *Gryllotalpa vulgaris*, but, as stated in the discussion of the spermatogonia, his results are so evidently erroneous that it is profitless to attempt a comparison between them and the findings of others. It is only necessary to mention, to indicate the character of his work, that he reported the diploid number of chromosomes to be 12 instead of 23, and that he entirely overlooked the prominent accessory chromosome. For these reasons his work will not be considered further.

The fact that the spermatogonial number of chromosomes in *Gryllotalpa borealis* is 23 is no reason for concluding that the spermatogonial number in *Gryllotalpa vulgaris* is 23. While I admit that vom Rath's figures are schematic and inaccurate,

my observations confirm his count of the spermatogonial number. The spermatogonial number in the material collected at Naples shows 15 chromosomes. Here we have at least two species and perhaps three in the same genus in which the chromosome number differs. It demonstrates clearly, I think, the fallacy of the argument that all closely related species must have the same number of chromosomes. It further shows the danger of generalizations.

7. ADDENDUM

As my paper was ready for the press, I received the final paper of Voïnov on *Gryllotalpa vulgaris*.³ His observations are very much as in his preliminary papers but more extensive. One difference should be mentioned. He finds 15 chromosomes in some spermatogonial cells, while 14 was the number previously observed. This number (15) agrees with my observations on the Naples material, although the size relations are not the same, as I find two small chromosomes instead of one. Voïnov collected his material near Bucharest and it is possible that the chromosomes in the material collected at Bucharest and Naples are different, as they were in the material collected at Naples and Freiburg. Even though our observations may have been made on different forms I cannot agree with his interpretations. He finds seven chromosomes in the first spermatocyte division. One of these seven is a small bivalent which he calls the m-chromosome. He believes this to be the same chromosome as the small one in the spermatogonial group. Hence he is driven to the assumption that the small spermatogonial chromosome is double. There must be at least 16 spermatogonial chromosomes then. Another one of his first spermatocyte chromosomes is an unequal pair, which divides unequally. This he calls the XY pair. As there is only one small spermatogonial chromosome he is driven to make another assumption, that is, that the Y-chromosome must be present but not visible. This would make all told 17 spermatogonial chromosomes, two more than he has

³ Recherches sur la spermatogénèse du *Gryllotalpa vulgaris* Latr. Archiv de Zool. Exp. et Gen., Tome 54.

actually observed. But his difficulties do not end here. A third spermatocyte chromosome is L-shaped. This Voïnov believes to be made up of a bivalent and the accessory. It is purely a belief, however, as he offers no proof that such is the case. In division, he believes that the bivalent passes to one end of the spindle and the accessory to the other. As pointed out earlier, this is not the way we would expect such a chromosome to divide, even if it were a bivalent and an accessory. These three spermatocyte chromosomes (the L, XY, and m) account for seven of the 17 spermatogonial chromosomes.

There are still ten spermatogonial chromosomes to be gotten into four first spermatocyte chromosomes. Voïnov does this by assuming that four spermatogonial chromosomes unite to form a large spermatocyte chromosome, which divides regularly.

Here are four assumptions made by Voïnov, which I believe to be needless, as the facts can be explained in an easier and better way. He even attempts to bring my observations on *Gryllotalpa borealis* in line with his. Voïnov's first assumption is that the small spermatogonial chromosome is bivalent and that it forms the m-chromosome of the first spermatocyte. According to my observations on *Gryllotalpa borealis* the single small spermatogonial chromosome forms the small end of the unequal pair. This is a perfectly logical conclusion, and we are not forced to assume that the small spermatogonial chromosome is a bivalent and that another small chromosome exists but is invisible. His third assumption is that the L-chromosome is a bivalent plus an accessory. Such may be the case, but this chromosome divides into two equal parts, a thing which we would not expect it to do if it were composed of a bivalent and an accessory. His fourth assumption is that four spermatogonial chromosomes fuse to form a large bivalent which behaves regularly. By making these assumptions he gets his 17 spermatogonial chromosomes (two of which he has not seen) into the seven spermatocyte chromosomes.

As previously stated my observations on the material collected at Naples show there are 15 chromosomes in the spermatogonial group, and it indicates there are eight first sper-

matocyte chromosomes instead of seven. If such be the case, the same explanation holds here as in *Gryllotalpa borealis*. Two of the spermatogonial chromosomes, unequal in size conjugate to form the unequal pair. The L-chromosome is an ordinary bivalent, the single unpaired chromosome remains single, and the other ten spermatogonial chromosomes pair two by two to form the five bivalents. This explains all the facts without making any assumptions. Further, my conclusions in *Gryllotalpa borealis* are backed by the oogonial counts which show 24 chromosomes, none of which are small.

A paper by Wenrich has also just been received.⁴ I wish merely to call attention to his postscript added after having read Voïnov's preliminary paper on *Gryllotalpa*. I quote as follows:

Unfortunately I had overlooked the results reported for *Gryllotalpa vulgaris* by Voïnov ('14), who found in the first spermatocyte metaphase an unequal pair of dyads, which separate so that sometimes the larger dyad and sometimes the smaller one goes to the same pole as the accessory chromosome. These results are in accord with those mentioned above for the Acrididae and the Tettigidae and it may be surmised that similar conditions perhaps obtain for *Gryllotalpa borealis* but have so far been overlooked.

My reason for quoting this is to show how readily Wenrich accepts Voïnov's observations and interpretations, without any hesitation or study, because they fall in line with his own work, and at the same time how readily he discards my own because they do not agree. I believe that my figures in the present paper will be convincing to any one who will take the trouble to study them with an unprejudiced mind. Because certain results agree or disagree with our own preconceived ideas, is no reason for accepting or rejecting them. It seems to me it is time we were realizing that evolution in chromosomes as morphological units, in chromosome numbers, and in chromosome behavior has been as diverse as it has been in external morphological characters. It would be about as logical for me to conclude that

⁴ The spermatogenesis of *Phrynotettix magnus* with special reference to synapsis and the individuality of the chromosomes, Bull. Mus. Comp. Zool. Harvard, vol. 60.

the chromosomes of all species of Orthoptera behave in the same way, as to conclude that all fishes of an order must have ten spines in the dorsal fin because six of them have that number.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Gryllotalpa borealis

A Spermatogonial cyst showing mitochondria in the inner end of the cell.

B The early growth period showing mass of mitochondrial granules and chromatoid body lying in the cytoplasm. Two other bodies are also present in the cytoplasm.

C A mitochondrial mass from the early growth period showing a central spherical body.

D The nucleus, mitochondria and chromatoid body in the prophase of the first spermatocyte division.

E and F Metaphase plates, side views of the first spermatocyte division, showing the mitochondrial threads.

G and H First division, metaphase plates, showing the arrangement of the mitochondrial threads around the spindle.

I and J Two late anaphases of the first spermatocyte division. In I, the mitochondrial threads have divided; in J, they have moved to the end of the spindle. The filar arrangement here seems to be lost, due in part, perhaps, to the fact that the slide was destained more than usual.

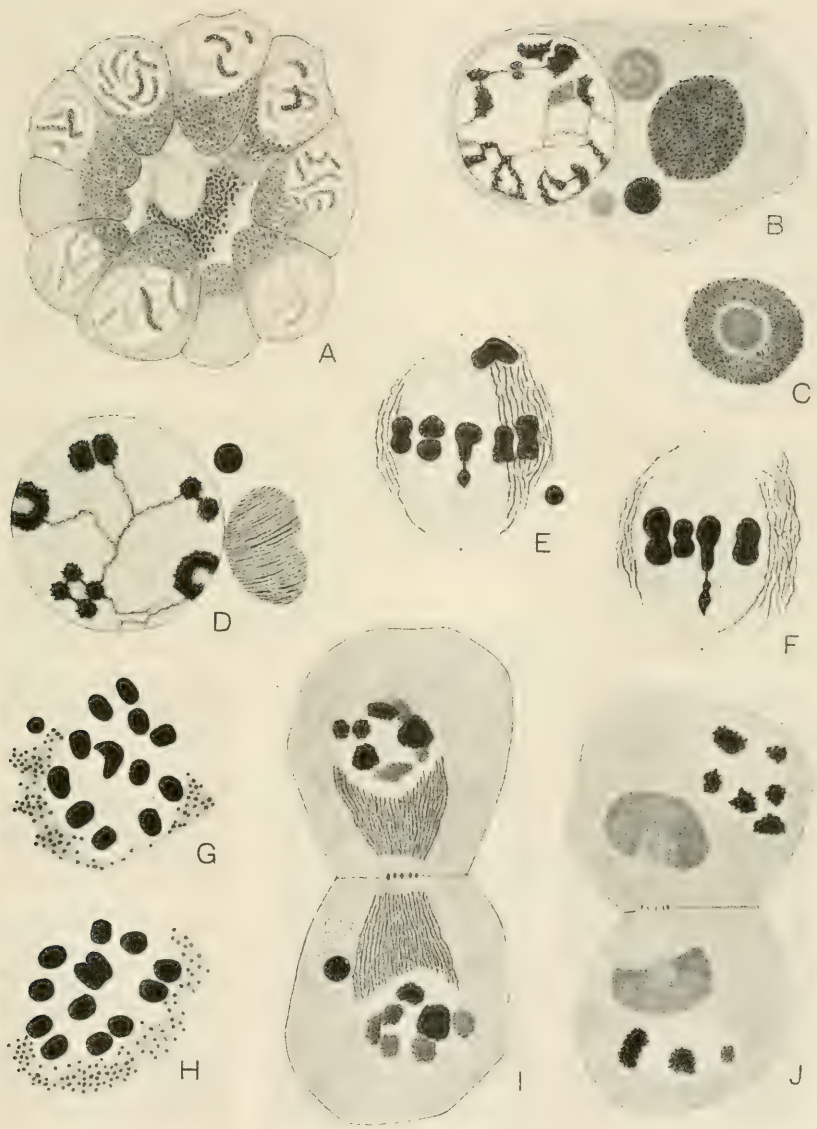


PLATE 2

EXPLANATION OF FIGURES

Gryllotalpa borealis

A Anaphase, side view, second maturation division, showing arrangement of mitochondrial threads on the spindle.

B Late telophase, second maturation division. The mitochondrial threads have again divided and moved to the ends of the spindle.

C A spermatid before transformation begins.

D and E An early stage in the transformation of the spermatid. The elongated body in contact with the nucleus and the spherical body were not present in C.

F A later stage than D and E. The mitochondrial threads have changed and the elongated body has moved to the opposite side of the nucleus.

G, H, I, J, K Still later stages in the transformation of the spermatid. G shows the beginning of the axial filament.

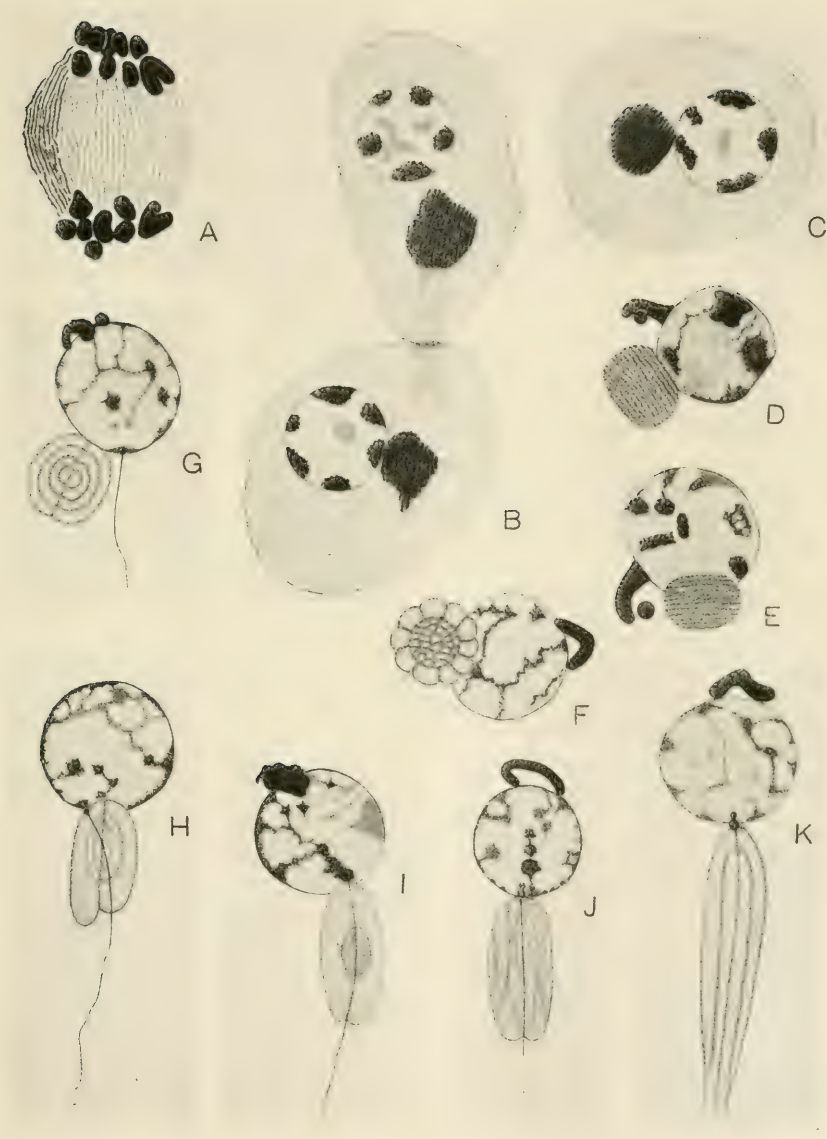


PLATE 3

EXPLANATION OF FIGURES

Gryllotalpa borealis

A to Q Further transformation of the spermatid into the spermatozoon. A and B are two views of the same stage. G is a cross section of the mitochondria and axial filament in the same stage as K. I is a view of the middle-piece at right angles to H. J shows the wave like movement of the cytoplasm along the tail. L shows the axial filament extending beyond the cytoplasm. M, N, O, P, show the elongation of the nucleus. Q is a mature spermatozoon. Sometimes the acrosome stains dark like the nucleus. In the final stages the mitochondria and axial filament seem to lose their identity.

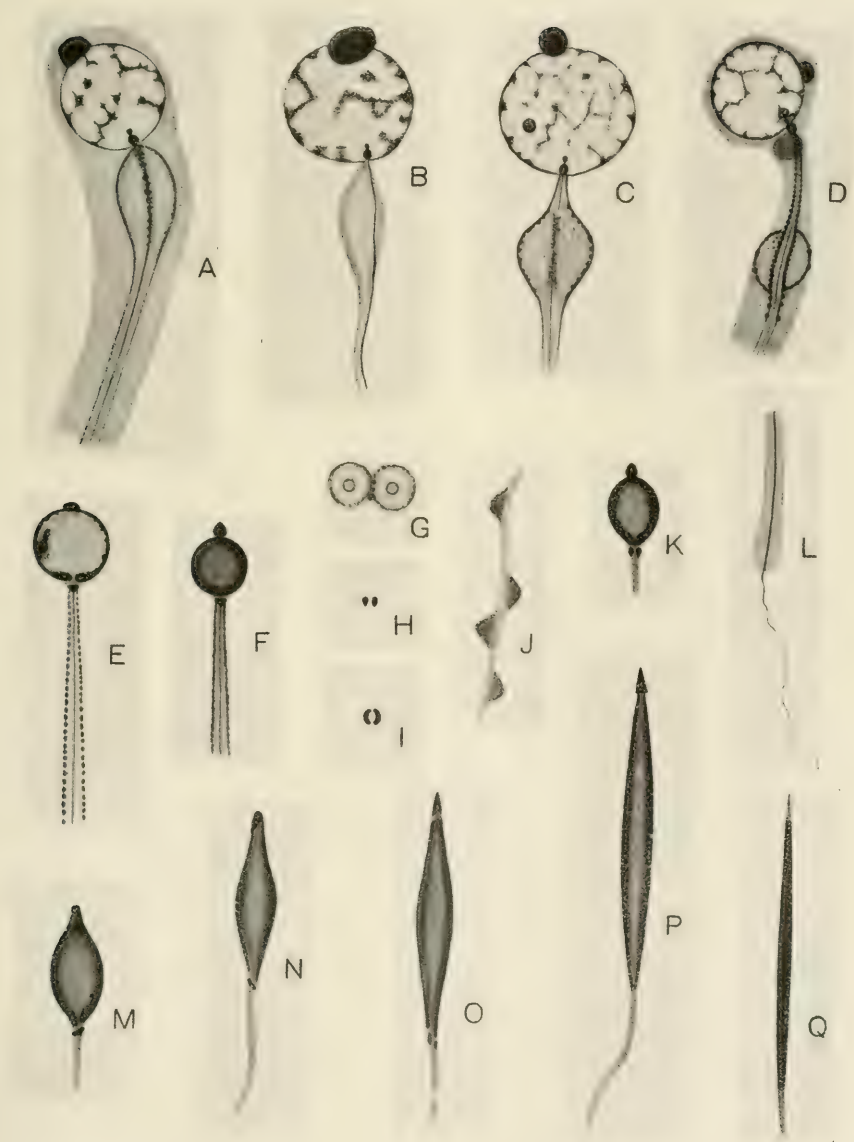


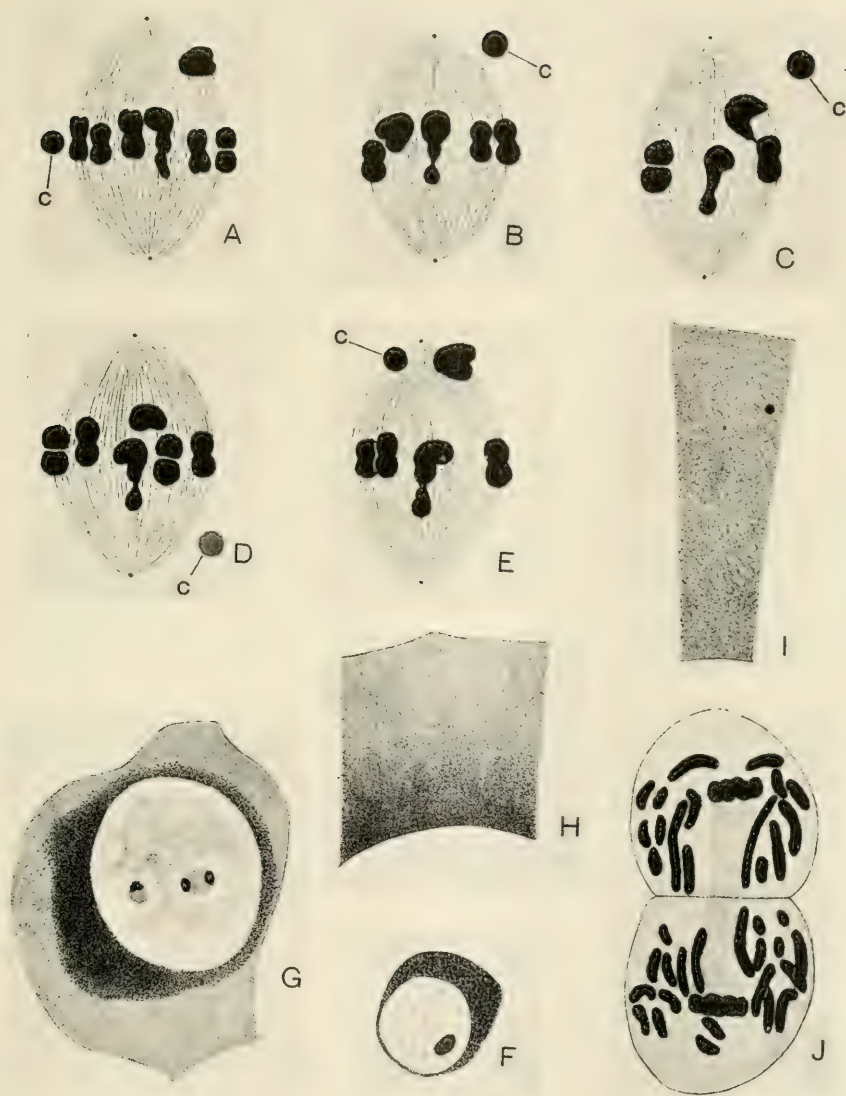
PLATE 4

EXPLANATION OF FIGURES

A to E *Gryllotalpa borealis*, side views, metaphase plates of the first spermatocyte division, showing the single unpaired chromosome and the chromatoid body.

F, G, H, I *Gryllotalpa vulgaris*, Naples. F, an oogonial cell with mitochondria. G, H, I, Stages in the growth period showing migration of mitochondria out into the cytoplasm.

J *Pyrrhocoris apterus*, first spermatocyte division, late anaphase, showing the marked difference in the appearance and arrangement of the mitochondria when compared with *Gryllotalpa*.



ON THE DEVELOPMENT OF THE DIGITIFORM GLAND AND THE POST-VALVULAR SEGMENT OF THE INTESTINE IN *SQUALUS ACANTHIAS*

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THIRTY FIGURES

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THE DIGITIFORM GLAND

Introduction

The results here presented were nearly completed in 1914 but laid aside temporarily for another research. An abstract of the first part of this paper has already been published (Hoskins '15). The work was made possible through access to a very complete serial collection of *Acanthias* embryos obtained in part from the Harpswell Laboratory, and belonging to Dr. R. E. Scammon, and to others secured from the Harvard Embryological Collection through the kindness of the late Dr. C. S. Minot. I desire here to thank Dr. Scammon for suggesting this investigation, for the loan of material, and for many helpful criticisms.

The terminology of the digitiform gland has been much confused. Alexander Monroe *secundus* (1785) seems to have been the first author to give a name to the gland in question. He referred to it as the appendix digitiformis, but did not describe it. The structure has also been termed the bursa cloacae (Retzius '19), the Darmanhang (Carus '34 and Gegenbaur '78), the caecal appendage (Owen '46), the superanal gland (Blanchard '82) and the anal gland (Crawford '99). Mention of the structure is found in a large number of early works on comparative anatomy, as for example, Cuvier (1810), Blainville (1811), Home (1814), Rathke ('27), Leydig ('52), Hyrtl ('58), Stannius ('54), Dumeril ('65), and others. It has been seen in all selachians. Glandular elements which seem to resemble the digitiform gland are found in the wall of the intestine of *Chimaera* (Mazza et Perrugia '94, Morgera '10).

Blanchard ('78a, '78b) gave the first account of the development of the digitiform gland. He found the gland in an *Acanthias* embryo 23 mm. in length, developing from a groove on the left dorsal intestinal wall. The 'glandules' are embedded in connective tissue which penetrates to its internal surface. Each 'glandule' is surrounded at its mouth by smooth muscle. In Blanchard's figures 7 and 8 ('78a), the main lumen of the gland is pictured as lined with connective tissue for which he seems to have mistaken the stratified epithelium. Blanchard's statement that the gland develops on the left side of the intestine is incorrect, as its origin is always from the right side.

Saufelice ('89a, '89b) noted the digitiform gland in *Pristiurus* and *Torpedo* embryos, 15 mm. in length. In embryos 19 to 20 mm. long the gland had lengthened, the epithelial cells were cylindrical with regular nuclei. The last stage described (30 mm.) showed many outpouchings from the central lumen.

Hoffman ('93) reported that in *Acanthias* and *Mustelus* embryos up to 26 mm. in length no blood vessels exist in the digitiform gland. They were found in earlier stages in the present investigation.

Scammon ('11) stated that the digitiform gland of *Acanthias* appears first in embryos of 18 mm. in length, as a solid bud on

the dorsal surface of the intestine, just posterior to the spiral valve. He has since called my attention to the fact that it appears as a hollow bud and is indicated in an embryo of 15 mm. as shown in figure 1 in the present paper. He traced its growth through several stages to embryos of 37 mm. in length.

Kearney ('14), working with *Mustelus*, stated that "the relative weight of the rectal gland appears variable in the embryo, but falls from an average of 0.105 per cent to about 0.032 per cent at birth. Shortly after birth the relative weight increases slightly and thereafter decreases."

Kellicott ('08) stated that at birth the relative weight of the digitiform gland in *Mustelus* was found to be 0.0398 per cent of the body-weight. He found no difference between sexes in the weight of the gland in most cases. In 9 female and 9 male specimens of *Mustelus*, averaging 73 cm. in length, examined by the present writer, the digitiform gland of the females averaged 23.5 mm. in length and in the males 22.5 mm. This difference is within normal variability.

Blanchard ('78 a, '78 b) presents the first adequate histological description of the digitiform gland, although Leydig ('52) reported briefly on its microscopic structure, analogizing it with the acinous glands of Brünner. According to Blanchard, the gland in question shows the following characteristics. The duct usually opens into the intestine in the mid dorsal line, through an opening guarded by a fold of the mucosa. A section through the middle of the gland shows the surrounding connective tissue capsule which is continuous with a heavy septum which extends to the central lumen. The parenchyma is not acinous but tubular, each tubule having many branches which end in a culs-de-sac. The glandular tissue is lacking where the duct joins the intestine.

Pillet ('85) stated that the gland is covered by peritoneal epithelium, under which are the round culs-de-sac of the tubules which resemble the glands of Lüberkuhn. The tubules are lined with simple cuboid epithelium. Cell boundaries are indistinct. The large, centrally placed nuclei contain three or four nucleoli. The cytoplasm consists of rather fine pale gran-

ules arranged in rows. At the center of the gland the connective tissue pushes the wall of the lumen into villous-like processes covered with 'calciforme' epithelium which also descends a short distance into the tubules.

Sanfelice ('89) noted smooth muscle in the capsule of the digitiform gland. He described the epithelium of the central lumen and of the ducts (tubules) emptying into it as stratified squamous and that of the tubules (parenchyma) as glandular. He noted goblet cells in the wall of the main central lumen and excretory ducts.

Crawford ('99) divided the digitiform gland histologically into three layers, namely, an outer fibro-muscular layer covered with peritoneum, a middle glandular layer, and an inner region of ducts and blood vessels surrounding a central lumen. The outer layer consists of connective tissue and smooth muscle, both circular and longitudinal, and contains large sinuses. The glandular tissue is composed of tubules lined with granular, cubical, zymogen-free epithelium. In the inner layer the ducts and the irregular central lumen are lined by transitional epithelium, which is composed of several layers of polygonal cells flattened at the free surface. The outer layer is often degenerated. Among the ducts are large sinuses. The cells in the 'acini' (peripheral), judged from their resemblance to renal epithelium, may be excretory. The vascular system consists of central and peripheral sinuses joined by capillaries.

Sullivan ('07) noted that the surface cells lining the main central lumen undergo mucoid changes and that clear cells may be seen lining the ducts.

Pixell ('08) added that the tubules may turn at their peripheral ends and extend longitudinally, and that goblet cells are present in the tubules. She states that the digitiform gland resembles in structure the compound pyloric appendage of *Aci-penser* and *Lepidosteus*.

Hyrtil ('58) termed the artery from the aorta supplying the digitiform gland the posterior mesenteric. Parker ('87) called this artery the 'spermaticomesenteric posterior.' Hochstetter ('88) pictured the digitiform gland as though its vein enters

the junction of the two posterior cardinal veins in Raja, and the genital sinus in Torpedo. Howes ('91) stated that the artery to the digitiform gland is the inferior (posterior) branch of the superior mesenteric group. Neuville ('01, fig. 6, Acanthias, and fig. 9 Raja) pictured the dorsal intestinal artery and vein as terminating on the digitiform gland.

The functions attributed to the digitiform gland are nearly as numerous as the descriptions of it. Home (1814) likened it to the ink bag of cuttle-fish and to the intestinal caeca of birds. Leydig ('52) stated that it resembles the acinous glands of Brünner. Hyrtl ('58) thought it accessory to the reproductive system. Milne-Edwards ('62)—(Howes '91 to the contrary)—referred to it as a urinary bladder. Dumeril ('65) stated that the digitiform appendix is a true organ of secretion. Blanchard ('82 a and '82 b) obtained from the digitiform gland extracts able to digest starch and emulsify fats but which had no action on proteins. He considered the gland analogous to the circumanal glands of some higher forms. He stated that it probably does not function in digestion although potentially able to do so. Howe's ('91) argued from its blood supply that the gland may be considered as similar to the vermiform appendix and caecum of higher animals. Crawford ('99) obtained the urea reaction with the substance found within the gland. Wiederstein ('07) thought that it might correspond to the caecum of higher forms. Sullivan ('07) stated that the digitiform gland has no digestive activity. Pixell ('08) obtained a starch and fat-splitting enzyme from the digitiform gland by a sodium carbonate extraction. Morgera ('16) stated that the gland may have an internal secretion which contracts the intestine.

Development of the digitiform gland

External form. Half way between the spiral valve and cloaca on the right dorso-lateral surface of the intestine in an Acanthias embryo of 15 mm. there appears a very slight bulging of the wall which may be called the area of evagination, or anlage, of the digitiform gland (fig. 1). The alteration of the part of the intestine concerned in this formation is rather diffuse,

involving a relatively large portion of the side and roof of the gut. The bulging is barely distinguishable on the external surface, but is evident in sections. There is no thickening of the epithelium in the anlage of the digitiform gland.

In embryos of 19 mm. the area of evagination is more sharply marked off from the rest of the gut and is relatively smaller. It forms a shallow hollow bud (fig. 21) which projects laterally from the intestine.

The gland, once established, grows with rapidity, assuming at first the form of a definite bud, and later becoming tubular (fig. 22). In an embryo of 22.5 mm. it is 0.26 mm. long. The distal extremity is turned anteriorly. The external surface of the anlage is still smooth at this stage, with no indication of secondary outgrowths. As the length of the gland increases it pushes cephalad along the right dorso-lateral surface of the intestine although touching that structure only at the proximal end.

Owing to the rapid growth of the proximal portion of the gland, which later becomes the duct, and to the fact that the distal portion does not move forward sufficiently rapidly to keep the structure extended, the proximal part soon bends posteriorly as seen in figure 23, of a 28.1 mm. embryo. In all later stages the proximal part (or duct) of the gland first extends backward and then bends anteriorly. In the 28.1 mm. stage the posterior division of the gland is 0.20 mm. in length, or one-fifth of the entire length of the gland. The outer surface of the gland (fig. 27) has become irregular in outline, owing to the beginning of primary tubule formation.

In the 33.1 mm. stage (fig. 24) the proximal portion (or duct) of the gland is shorter than in the 28.1 mm. embryo, being but 0.12 mm. long, but the entire length of the gland has increased to 1.3 mm. The duct is bent sharply. The outer surface is very irregular, owing to the great increase in the number of primary tubules, which are actually smaller than in the preceding stage (fig. 28). They have assumed various shapes, owing to crowding, but most of them are flattened at the periphery from the pressure of the capsule. Some of the tubules

are nearly cuboid in form. Some of the primary tubules have begun to form tubules of the second order.

In a 47.5 mm. embryo (fig. 25), the external surface of the (entodermal) digitiform gland has undergone considerable change. The general shape is still cylindrical with narrowed extremities. The duct has decreased in diameter, but is of greater length. It extends posteriorly and laterally for nearly a millimeter, and curves around a bend in the intestine in its lateral course. The ostium of the duct is about the same distance from the spiral valve as in the 22.5 mm. embryo, although the intestine has nearly doubled its length in this region. The diameter of the gland is about twice that of the 33.1 mm. embryo, and the number of tubules has more than doubled. The newly formed primary tubules (fig. 29) are much smaller than those of the 28.1 mm. stage (fig. 27). Many of the original primary tubules are still large and unbranched, but others have branched in various directions, forming secondary tubules of various shapes and sizes. This branching is dichotomous in places, but also there are clumps of small tubules of the second order formed from a single tubule of the first order.

In the embryo 95 mm. long (fig. 26) the digitiform gland and duct together are about 6.6 mm. in length. The duct is about 1.5 mm. long and extends from the gland in an oblique plane, running backward for a short distance, then turning sharply away from the intestine. Almost the entire duct is embedded within the wall of the mesenchyma of the intestine. Its end, which is solid, joins a solid ridge of the gut (fig. 13). The lumen of the duct and intestine are continuous through a lateral slit-like opening in the duct (fig. 14). The internal surface of the gland is very irregular, owing to the development of a large number of tubules (fig. 30). As many as fifty tubules may appear at the periphery in a single transverse section of the gland. These extend radially from the longitudinal axis and nearly at right angles to it, although some are turned toward the proximal end of the gland. Some of the primary tubules are more or less isolated from the others, but many tubules are grouped in somewhat cone-shaped clusters, the

apex pointing toward the center of the gland. The region between adjacent apices contains many small tubules which have branched nearly at right angles from the primary tubules, and a few small primary tubules which have developed from the wall of the main lumen. The tubules in this stage branch in nearly every possible manner. Some primary tubules extend unbranched to or nearly to the periphery, some divide dichotomously near the center of the gland into secondary tubules which extend unbranched to its surface. Others give off several secondary tubules at right angles to their straight radial course, and still others dilate suddenly near their origin and branch into as many as five secondary tubules at the same place. The secondary tubules branch in much the same way as those of the first order, giving off tubules of the third order which, in turn, branch to form tubules of the fourth order.

In the 200 mm. *Acanthias* the duct of the digitiform gland runs backward in the sub-mucosa for about 3.0 mm. from its opening into the intestine. It then turns obliquely and penetrates the muscular layer, forming a ridge on the right dorso-lateral surface of the intestine and finally extends cephalad free from the intestine. The gland lies dorso-lateral to the intestine. The length of the gland varies considerably, but it may be over 20 mm. long. In general shape it is cylindrical, but both extremities are tapering. The number of tubules has increased considerably over that of the preceding stage, tubules up to the sixth order being present.

In the adult the gland has much the same shape as that just described. The length has a large variability, sometimes being only 2 or 3 cm., and again it may be more than 7 cm. in length. The adult gland (fig. 10) contains tubules similar to those described above, except that they are longer and of greater diameter. Their number has increased greatly. In a single transverse section of the gland over 600 tubules can be counted at the periphery, and many thousands are present in the entire gland. Around the central lumen of the gland are two to five rows of primary and secondary tubules, extending both radially and longitudinally. The next region peripheral to this and about

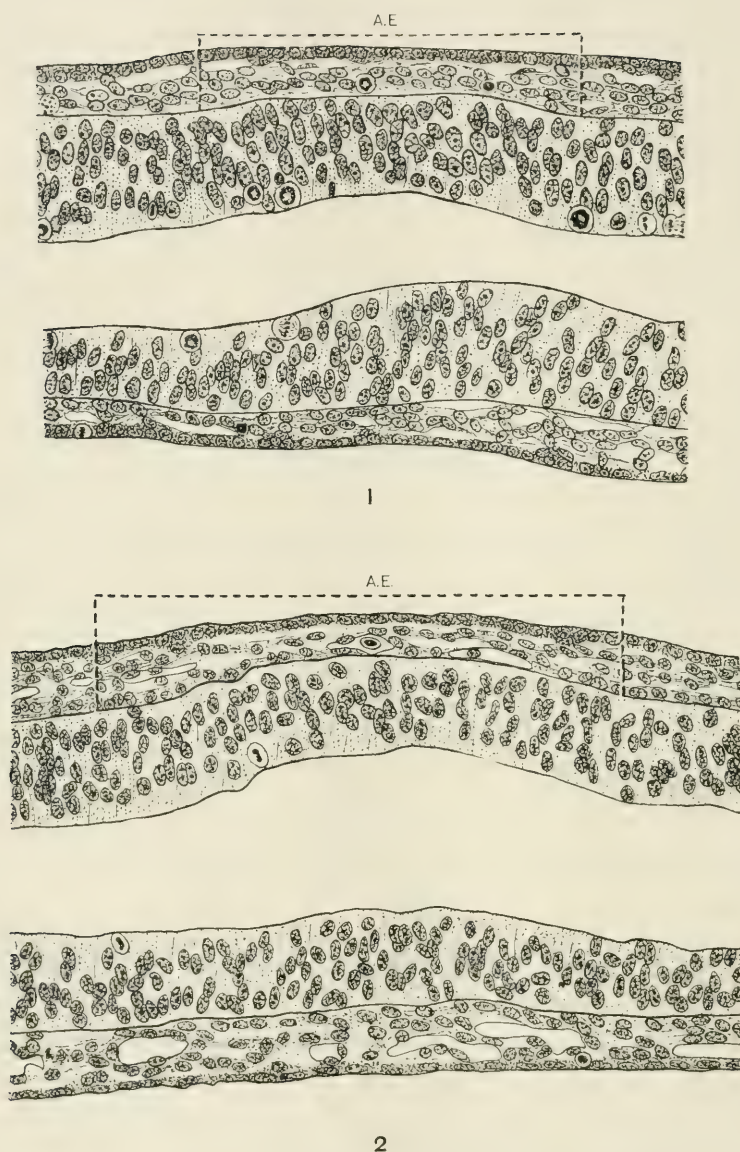
one-half as wide as the radius of the gland, contains radial tubules of the second, third, fourth and fifth, but mostly of the third and fourth order. The peripheral two-sevenths of the gland contains tubules of all six orders but mainly of the fifth and sixth. At the periphery some of them end in points, some are dilated and others bend to run a short distance longitudinally. Most of the tubules in the adult gland are about $60\ \mu$ in diameter except at a junction of two or more tubules or at dilated extremities.

Cytology. The earliest stage drawn (fig. 1, 15 mm.) shows no cellular changes in the area of evagination as compared with the rest of the post-valvular intestine, beyond a slight condensation of nuclei, and a slight rounding of many of them. Cell boundaries are indistinct, but four or five rows of nuclei may be counted. The surrounding mesenchyma contains more lightly staining oval nuclei and in the serosa the nuclei are dark and nearly spherical. A few endothelial lined vessels are seen in the mesenchyma.

In the anlage of the digitiform gland in the 19 mm. embryo (fig. 2) many of the nuclei are round and show change of position. The longest axis of many of the elongated nuclei is parallel with that of the intestine. The nuclei in this stage are relatively fewer in number than in the 15 mm. stage, but are of about the same size. The lateral bulging of the wall of the gut has flattened the mesenchymal cells covering the anlage of the gland. The mesothelium is likewise flattened in this region, its nuclei being oval in shape. Vascular channels are numerous.

In the 22.5 mm. stage the epithelium of the digitiform gland is still composed of four layers of cells as shown by the nuclei, the cell boundaries being indistinct. At the rapidly growing, distal end of the gland, most of the nuclei are round, but toward the gut they appear oval in outline. The anlage is surrounded by flattened mesenchymal cells covered with a thin peritoneal layer. The lumen is still smooth and regular.

As shown by figures 3 and 7 of an *Acanthias* embryo of 28.1 mm. tubule formation is well under way. These evaginations are relatively very large, involving often as much as a fifth of



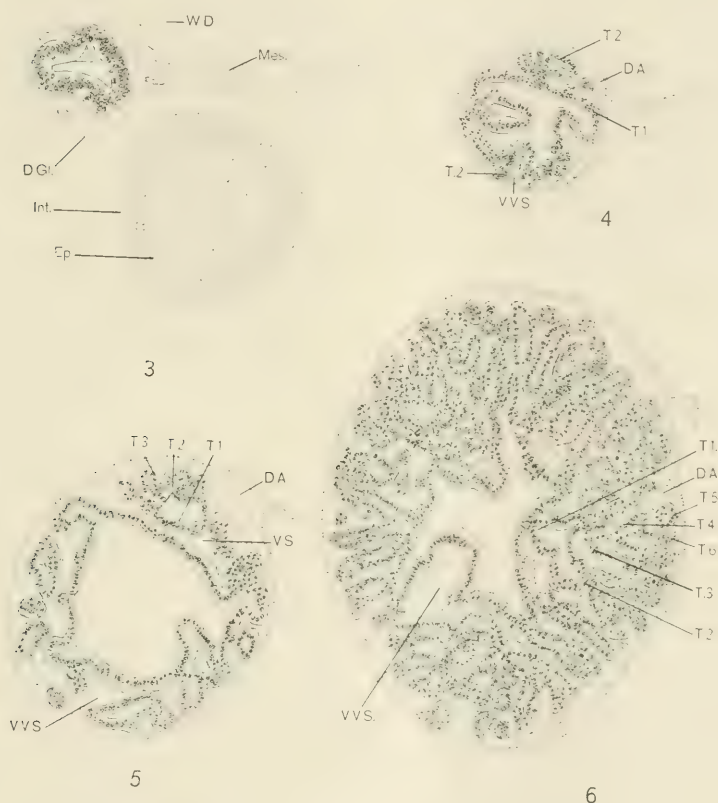
Figs. 1 and 2 $\times 200$. Longitudinal sections of the right dorso-lateral wall of the post-valvular intestine through the anlagen of the digitiform gland of *Squalus acanthias* embryos.

Fig. 1 Embryo 15 mm. in length (H. E. C. 229).

Fig. 2 Embryo 19 mm. in length (H. E. C. 138).

A.E., area of evagination of digitiform gland.

the periphery of the gland as seen in transverse section (fig. 3). They resemble closely the original outpouching of the gland from the intestine. At the peripheral ends of the newly forming tubules the epithelium is reduced to one or two layers of



Figs. 3, 4, 5, and 6 $\times 80$. Transverse sections through the middle of the digitiform gland of *Acanthias* embryos.

Fig. 3 Embryo 28.1 mm. in length.

Fig. 4 Embryo 33.1 mm. in length.

Fig. 5 Embryo 47.5 mm. in length.

Fig. 6 Embryo 95 mm. in length.

Cent. lum., main central lumen; *D.A.*, digitiform artery; *Ep.*, epithelium; *Int.*, intestine; *Mes.*, mesentery; *T. 1, 2, 3, 4, 5, 6*, tubules of the first to sixth order; *V.S.*, venous sinus; *V.V.S.*, ventral venous sinus (digitiform vein).

Only the larger vessels are shown. In figure 3 note the narrow lumen of the intestine.

cells, whereas in the wall of the gland between the tubules there are three or four layers. Here, as in all the other stages, the peripheral, actively growing, and therefore young cells are round or nearly so, and contain large round nuclei and relatively little



7



8

Figs. 7 and 8 $\times 400$. Transverse sections through the middle of the digitiform gland of *Acanthias* embryos, showing tubule formation and cytomorphosis.

Fig. 7 Embryo 28.1 mm. in length.

Fig. 8. Embryo 95 mm. in length.

Only four orders of tubules are shown in figure 8, although in some parts of the gland in this stage six orders of tubules may be found (see fig. 6).

cytoplasm. In the older, central part of the gland the cells are columnar and contain oval nuclei, and relatively more cytoplasm. The lumen of the gland is narrow, even slit like in places, owing to the fact that the entire wall is involved in the tubule formation. The surrounding mesenchymal and peritoneal cells are rather flat, with oval nuclei. There is no certain differentiation of smooth muscle. The proximal portion of the gland is lined with columnar epithelium showing two to four layers of nuclei.

Still greater changes are to be seen in the 33.1 mm. stage (fig. 4). The number of tubules of both the first and second orders has greatly increased. The main wall of the gland is thinner than before and contains relatively fewer and smaller nuclei. In most places, not only in the tubules but also in the wall of the main lumen, there is but a single layer of cells. As noted before, the younger nuclei are round and the older are oval in outline. The lumen of the gland is irregular and its inner surface is much pitted. The proximal portion (duct) of the gland is lined with an epithelium which is narrower than in previous stages. It consists of but one or two layers of columnar cells with oval nuclei. The outer mesenchymal cells show fibril formation and their nuclei are flattened and elongated. The mesothelial outer covering is quite thin.

Few cellular changes are to be seen in the digitiform gland of the 47.5 mm. embryo (fig. 5) except that the epithelium is thinner and the nuclei are relatively smaller. The surrounding mesenchyma and mesothelium have become more compact. Numerous new tubules have formed, but, in finer structure, they do not differ from those already described. Vascular channels are numerous. The lumen of the gland is relatively large. The duct is lined with high columnar cells.

In the 95 mm. embryo (figs. 6 and 8), the columnar epithelial cells in the tubules, as well as in the wall of the lumen, are relatively larger and contain smaller and fewer nuclei. The newly formed tubules of the third and fourth order are of about the same diameter as those from which they have arisen, except at the distal ends which are narrower. Both connective tissue and smooth muscle nuclei may be made out in the proximal

portion of the gland (fig. 8). The loose connective tissue among the tubules contains numerous blood vessels. The lumen of the gland is very irregular, the inner wall of the gland containing many pits and crypts into which the primary tubules open individually and in groups of two to five.

The duct, as before, is lined with high columnar granular epithelial cells. Its surrounding covering is well differentiated into connective tissue, smooth muscle and serosa, all of which become continuous with the corresponding layers of the intestine at the junction of the duct and gut.

The peripheral ends of the tubules of the 200 mm. stage are composed of cuboid or rounded cells with round nuclei. Toward the central lumen the epithelium gradually increases in height, becoming columnar in type, with elongated nuclei. The cytoplasm is finely granular, staining heavily with eosin. At the mouths of the primary tubules the epithelium undergoes transition into the stratified type which lines the main central lumen and central ends of the primary tubules. Where a primary tubule opens directly through the main wall of the central lumen, this change is very sudden, but where it opens into a pit in the wall the transition is more gradual. The stratified epithelium of the central lumen varies from two layers of cells in depressions in the wall to four or five layers on the more exposed places. Where the stratified epithelium is thick, the outer layer of cells is composed of quite flat cells with rounded free surfaces, resembling those seen in the urinary bladder of higher animals. Their nuclei are flattened and lie with the long axis parallel with the surface. The cells beneath the surface layer are for the most part irregularly rounded, although some are columnar and perpendicular to the inner surface of the gland. The former have round and the latter elongated oval nuclei. The epithelium illustrates the law that a nucleus tends to assume the shape of its cell. Here and there, in the surface layer of epithelium, are large isolated goblet shaped cells which give the customary reaction for mucus with Mallory's tri-color connective tissue stain. The loose connective tissue among the tubules is continuous with the dense layer in the capsule. The smooth muscle is very definite in the capsule below the serosa.

The duct extends some little distance into the proximal portion of the gland, running at first in the ventral half but more distally, taking the central position. A section through this region shows a structure, part gland and part duct, each with its characteristic epithelium. The epithelium of the duct near the intestine is of the stratified columnar type, containing, on the free border, very numerous goblet cells which gradually diminish in number toward the gland where only occasional goblet cells are found. The external layers of the intestine, namely, the serosa, outer longitudinal smooth muscle, inner circular smooth muscle and submucosa are continuous with corresponding layers of the duct of the digitiform gland at the junction of the duct and intestine, but further distally the layers of muscle gradually break up, first the longitudinal and then the circular layer, so that only small bundles of fibers and isolated cells are seen, scattered through the connective tissue.

Development of the blood vessels. When the anlage of the gland moves laterally it carries with it the blood capillaries in the wall of the gut (figs. 1 and 2). These are supplied through capillaries in the mesentery and are seen in all the stages studied (figs. 1 to 8). They arise from the aorta and form the digitiform artery. The capillaries in the gland penetrate among the forming tubules to the wall of the central lumen. Here they gradually form large longitudinal venous sinuses, as seen in the figures referred to above. In early stages (figs. 4, 5, and 8) the circulation seems to be of the sinusoidal type wherein large peripheral sinuses connect with centrally located sinuses by relatively large channels. This is the usual type of circulation found in the glands of *Acanthias* embryos. The sinusoidal circulation in the digitiform gland is lost, however, in its later development and there is present instead a peripheral arterial supply joined by capillaries to a central venous drainage system.

Development of the mesentery. In early stages the dorsal mesentery of the hind gut is attached along the intestine, from the spiral valve to the cloaca. As the digitiform gland develops and pushes laterally from the intestine (15 to 23 mm. embryos), it carries with it the intestinal mesentery, thus leaving a portion

of the gut free in the body cavity without a mesentery. This space between the intestine and the dorsal wall of the body cavity corresponds to the 'mesenteric fenestra' (Scammon '11). This fenestra increases in size and extent during the later growth of the embryo so that the gut lacks mesenteric support between the middle of the spiral valve and the entrance of the duct of the digitiform gland into the intestine. The mesentery is not

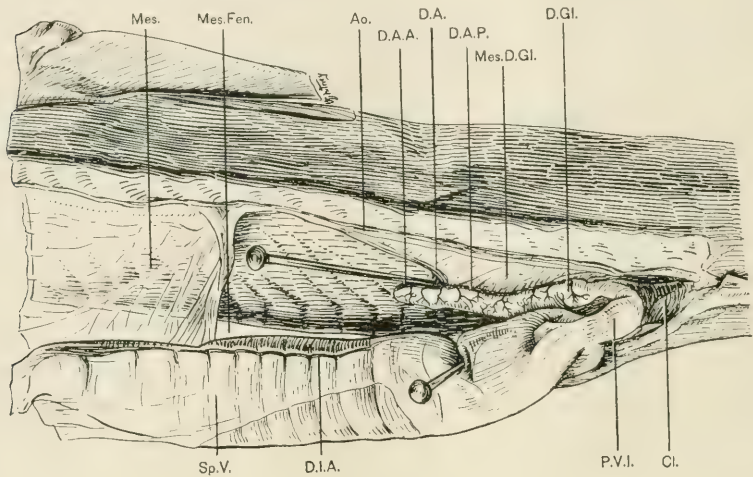


Fig. 9 $\times 1$. Dissection of a full term *Acanthias* embryo 220 mm. in length, drawn from the left side. *Ao.*, aorta; *Cl.*, cloaca; *D.A.*, digitiform artery; *D.A.A.* and *D.A.P.*, anterior and posterior rami of the digitiform artery; *D.Gl.*, digitiform gland; *D.I.A.*, dorsal intestinal artery and vein; *Mes.*, mesentery of intestine; *Mes.D.Gl.*, mesentery of digitiform gland; *Mes.Fen.*, mesenteric fenestra; *P.V.I.*, post-valvular intestine; *Sp.V.*, spiral valve.

The arteries on the digitiform gland were added to the drawing from another specimen in which the vessels had been injected.

really lacking, however, but attached to the digitiform gland, which thus is supported from the midline of the dorsal wall of the body cavity (fig. 9). The mesentery of the digitiform gland of the mature animal is thus quadrilateral in shape, with a long edge fastened to the dorsal body wall, a short edge fastened to the gland, a long oblique anterior border and shorter posterior border perpendicular to the longitudinal axis of the body (fig.

9). The anterior end of the gland extends beyond the mesentery for a short distance in most of the older specimens studied. In the adult the anterior third of the gland may lie free in the body cavity.

Histology of the adult gland

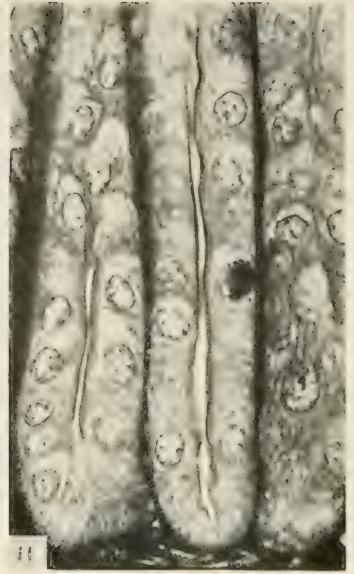
The histological structure of the digitiform gland of the adult selachian has been described in a large measure by various writers, hence it is unnecessary to give a lengthy description here (see Introduction, and Disselhorst '04). Attention may be called however to the accompanying figures 10, 11 and 12, which show the microscopic appearance of this peculiar gland.

As shown in figure 10, the gland is nearly circular in section. It consists of thousands of radial tubules grouped around a large irregular central lumen and surrounded by a narrow capsule of fibrous tissue and peritoneum. The sections shown in the figures 10, 11, 12, are from the middle segment of the gland. The digitiform artery lies at the base of the mesentery. Near the lumen, projecting into it, but under the epithelium, may be noted the ventral longitudinal venous sinus or digitiform vein. Between the gland proper and the capsule, in some sections, is a small group of isolated tubules which join the tubules of the gland proper at a different level. The capsule is composed of a serous outer covering of simple epithelium and an underlying connective fibro-muscular layer. The connective tissue is mostly of the white fibrous type. Among these fibers are both circular and longitudinal smooth muscle fibers but they are not in definite bands at this level. In other sections the muscle is seen in large bundles, the circular internal to the longitudinal. There are numerous nerves and capillaries in the middle of the capsule. From the capsule a septum of connective tissue enters the parenchyma.

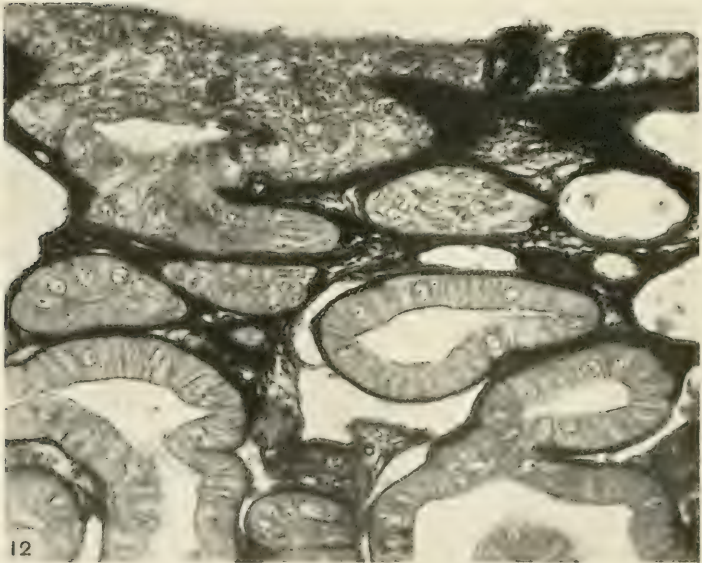
The parenchyma consists of tubules surrounded by connective tissue and capillaries (figs. 11 and 12) as has been mentioned above. The cytoplasm is very granular. The granules are in some places arranged in rows. In the cytoplasm near



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11



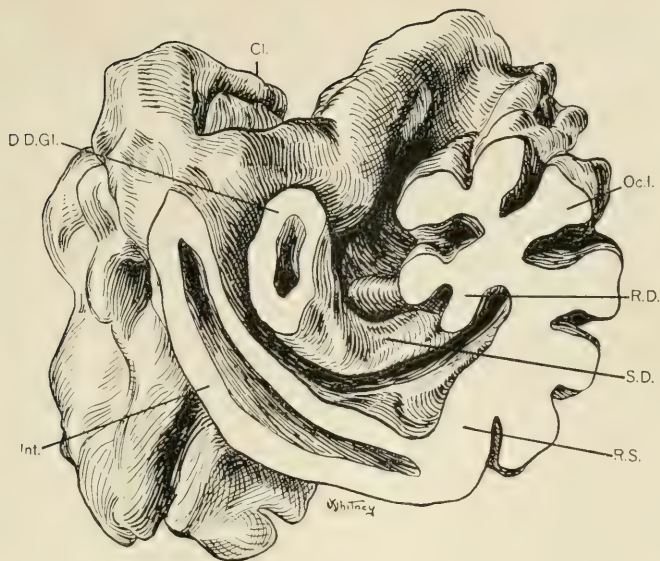
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Figs. 10, 11, 12 Photomicrographs of a transverse section of the digitiform gland of an adult *Acanthias*.

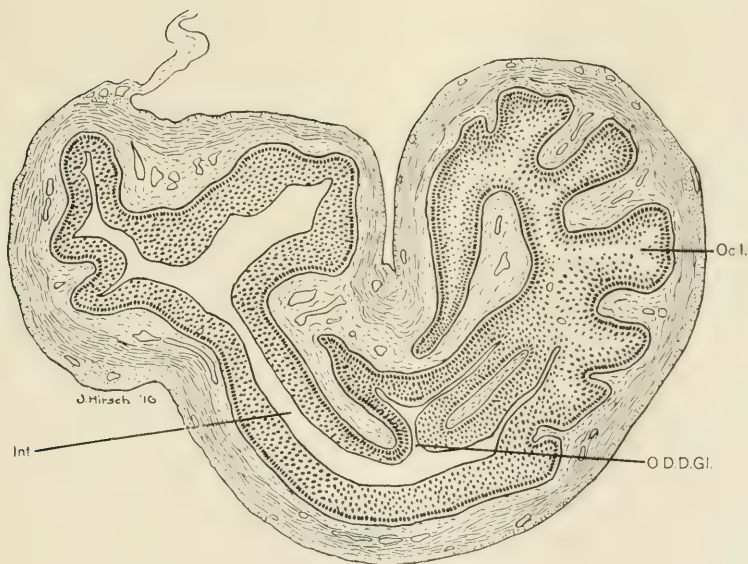
Fig. 10. $\times 10$, to show the general arrangement. Note the large venous sinus (digitiform vein) projecting into the central lumen.

Fig. 11 $\times 520$. To show a small portion at the periphery of the gland.

Fig. 12 $\times 280$ To show a portion at the center of the gland. Note the two large goblet cells at the right in the stratified epithelium which lines the central lumen.



13



14

Figs. 13 and 14 $\times 60$. Sections through the region of the ostium of the duct of the digitiform gland. Fig. 13, of the model (fig. 26) of the post-valvula intestine of an *Acanthias* embryo 95 mm. long, and fig. 14, a microscopic section at a lower level showing the junction of the intestine with the duct of the digitiform gland. *Cl.*, cloaca; *D.D.Gl.*, duct of digitiform gland; *Int.*, intestine with lumen into which the digitiform duct opens; *Oc.l.*, occluded portion of intestine anterior to digitiform duct; *O.D.D.Gl.*, ostium of the duct of the digitiform gland; *R.S.*, solid ridge which is continuous with the portion of the intestine marked '*Int.*' and which later splits to make the beginning of the lumen in the occluded portion of the gut; *R.D.*, solid ridge which is directly continuous with the solid end of the duct of the digitiform gland; *S.D.*, solid end of duct of digitiform gland.

the periphery of the gland there are light spots due to the fixation. Most of the nuclei lie parallel with the long axis of the tubule, although a few are at right angles to it. The nuclei always contain one very definite, and sometimes two or three, nucleoli. Many of the tubules are cut transversely near the central lumen (fig. 12) because they extend longitudinally for a distance. Smooth muscle nuclei are present in the connective tissue surrounding the tubules. The lining epithelium is stratified, composed of two to five layers of cells, the surface layer of which is squamous in some places, but in others resembles the surface layer of the transitional epithelium of the mammals. In this inner layer of epithelium are occasional goblet cells, most of which give the customary blue colored mucous reaction to Mallory's connective tissue stain, although some of them are filled with granules which are colored red with this stain.

Toward the proximal portion of the digitiform gland, as has been noted before (see 'Introduction'), the connective tissue increases in relative amount as compared with the parenchyma. The capsule is thickened by an increase in the smooth muscle which is very definitely arranged near the duct into an inner circular and outer longitudinal layer. The main central lumen gradually approaches the ventral surface. The number of mucous goblet cells increases in the inner epithelial layer and some are found also in the primary tubules.

The duct of the digitiform gland has the same layers and histological structure as the intestine, with the possible exception of more numerous goblet cells in the epithelium. These cells are so closely packed together in places that they are pushed out of their natural shape and position and give the appearance of being arranged in more than one layer. Near the junction of the duct and digitiform gland the former is composed of epithelium containing goblet cells on one side and glandular epithelium on the other. At the junction of the duct with the intestine the outer layers of the two become continuous, but the epithelial duct continues anteriorly, running in the submucosa of the gut, sometimes for nearly 2 cm., before it fuses with the epithelium of the gut, and the lumina of the two become con-

tinuous. This arrangement of the entrance of the duct is such that when the intestine is dilated the duct must be closed, unless the pressure within the gland and duct is rather high.

Blood supply. The digitiform artery arises from the aorta anterior to the digitiform gland and courses obliquely ventrally and posteriorly to the gland at or near to its distal extremity (fig. 9). It has been called the posterior (inferior) mesenteric artery by Parker ('87), and the posterior branch of the superior mesenteric by Howes ('91). It may branch in the mesentery, one ramus going anteriorly to the tip of the gland and the other posteriorly along the dorsal surface. The anterior ramus may be lacking. Numerous small rami are given off on either side from the digitiform artery on the dorsal surface of the gland (fig. 9). These surround the gland and anastomose on the ventral side, where a ventral longitudinal artery may be formed. Numerous capillaries from the surrounding arteries penetrate the parenchyma, so that each tubule is in contact with one or more blood vessels. These extend nearly to the central lumen and gradually anastomose, forming venous sinuses which run longitudinally along the lumen. The ventral sinus is the largest, and the others enter it before the duct is reached. This sinus (fig. 10) may be called the digitiform vein. It runs along the digitiform duct and empties into the dorsal intestinal vein (fig. 18). The blood supply is thus quite similar to that of the mammalian suprarenal body which has an external arterial supply and an internal venous drainage system. The rich blood supply suggests a secretory activity.

Nerves

The digitiform gland is well supplied with nerves (Monti '98). A large trunk which appears to contain both medullated and non-medullated fibers is present in the mesentery. It breaks up into numerous rami which may be found within the capsule of the gland. No ganglion cells were seen in any of the preparations examined. The ultimate distribution of the nerve endings was not made out because no special nerve stains were used.

Signs of degeneration were noted in various sections of the adult digitiform gland. Elongated cysts was found in the parenchyma. These apparently are formed from occlusion of the proximal part of tubules. They contain a substance which gives a mucous reaction to Mallory's connective tissue stain. They are bounded by a single layer of flat cells. A number of the cells in the parenchyma contain small vacuoles. Some of the surface cells in the central lumen, although having the shape of mucous goblet cells, contain large granules which stain red instead of the usual blue with Mallory's stain, and are colored red in hematoxylin and eosin preparations. In places the nuclei in the surface cells appear pyknotic.

Summary of Part 1

The digitiform gland appears in embryos of *Squalus acanthias* of 15 mm. in length as a general outpouching upon the right dorso-lateral side of the gut about midway between the posterior end of the spiral valve and the beginning of the cloaca. This anlage rapidly takes the form of a hollow bud which grows first toward the right then turns and grows anteriorly.

As the digitiform gland moves away from the intestine it carries with it the common mesentery, thereby leaving this part of the gut free in the body cavity, forming the 'mesenteric fenestra' between the intestine and dorsal wall of the body cavity.

The young gland forms outpouchings which extend laterally, at right angles to its longitudinal axis, and which resemble in form the original gland anlage. These outpouchings become the primary tubules and in turn give off secondary tubules.

By repeated subdivisions as many as six orders of tubules may be formed. The manner of tubule formation is unique. A primary tubule may extend unbranched to the periphery; it may divide dichotomously near the central lumen; it may extend nearly to the periphery, giving off several tubules at right angles to its long axis, but at irregular intervals, or, lastly, it may end near the central lumen, dividing suddenly into a cluster of as

many as five secondary tubules. The same is true, in general, of the secondary tubules, but those above the second order do not show this last type of division. The tubules may be very small or may be expanded at their peripheral ends.

The anlage of the digitiform gland consists of a stratified columnar epithelium of five or six layers of cells. This number very soon becomes reduced to two in the central lumen and to one or two in the tubules. Later, the epithelium of the central lumen and that of the first part of some of the primary tubules again becomes stratified. In the embryonic tubules beyond the primary tubules the epithelium is simple columnar with oval nuclei except at the growing peripheral ends where the cells are irregularly round with spherical nuclei.

In the adult these tubules contain three types of cells, (a) cuboid cells with round nuclei, (b) columnar cells with oval nuclei the long axis of which is at right angles to that of the tubule and (c) flattened hexagonal cells with oval nuclei, the long axis of which is parallel with the tubule. The peripheral ends of the tubules are often pressed against the surrounding tissue so that the cells there are forced into various shapes. The central lumen is lined with an epithelium much like that of the urinary bladder of higher vertebrates except that it contains mucous goblet cells. The duct of the digitiform gland in the adult has much the same structure as that of the intestine.

Blood vessels are present in the digitiform gland from the first. The digitiform artery arises from the dorsal aorta, anterior to the digitiform gland, and runs obliquely ventrally and posteriorly in the mesentery to the anterior extremity of the gland. It may or may not branch. It runs along the dorsal side of the gland at the base of the mesentery, giving off lateral rami which surround the gland. These may anastomose and form a slender vessel on the ventral side. From these arteries very numerous small capillaries enter the substance of the gland, penetrate among the tubules, and form from two to four longitudinal venous sinuses along the central lumen of the gland. The ventral sinus is the larger and the others empty into it toward the proximal end of the gland. This sinus extends along

the duct of the gland as the digitiform vein and empties into the dorsal intestinal vein. The circulation is sinusoidal at first. Later, capillaries are found instead of sinusoides.

Evidences of degeneration are present in the adult digitiform gland.

THE POST-VALVULAR SEGMENT OF THE INTESTINE

Introduction

Since comparative anatomists are not in agreement as to the terms to be applied to the different parts of the intestine between the spiral valve and the cloaca it seems proper to refer to this region of the intestine as post-valvular. As numerous descriptions of the intestine of young embryos already exist (Scammon '11), only the later stages need be considered here. Only that part of the intestine which develops from entoderm will be described in detail.

So far as the available literature shows, there is no description of the development of the post-valvular portion of the intestine in later stages of selachian embryos.

As to the embryonic occlusion of the digestive tube in these forms, several observations have been made. Balfour ('78) stated that in embryos of elasmobranchs the oesophagus is solid for a time. Blanchard ('78 b) mentioned that he found the gut to be solid in the region of the digitiform gland in an *Acanthias* embryo 23 mm. in length. Scammon ('11) gives the length of the solid portion of the oesophagus and hind gut in *Squalus acanthias* embryos in several stages. He noted the longitudinal grooves on the surface of the gut in this region.

It is well known that atresia of the intestine occurs normally in higher animals also, as for examples, in the hind gut of the chick (Minot '00) and in the human duodenum (Trandler '00, Johnson '10).

Development of the external form

In an *Acanthias* embryo of 15 mm. (fig. 20) the intestine, posterior to the spiral valve is somewhat cylindrical in form,

but tapers toward the posterior end, where it expands suddenly to form the cloaca. Its contour is fairly regular, though marked by several slight swellings, the largest of which will become the digitiform gland. At 19 mm. (fig. 21) the general shape is the same, but for the digitiform gland anlage and for a depression which occurs just anterior to it. At 22.5 mm. (fig. 22) the portion of the intestine between the spiral valve and the digitiform gland is slightly swollen; as compared with the previous stage and the digitiform gland is larger, but otherwise no changes of importance have occurred. The growth in diameter has been more rapid than the longitudinal growth. The post-valvular portion of the intestine (fig. 23) in an embryo 28.1 mm. in length is somewhat irregular in outline, the gut is constricted at the entrance of the duct of the digitiform gland and a few irregular longitudinal ridges and furrows are present. Two dorsal out-pouchings appear where the intestine joins the cloaca.

In the 33 mm. embryo (fig. 24) the portion of the intestine in question has undergone considerable change. The entire gut is flattened dorso-ventrally. In the region of the entrance of the digitiform duct, the gut is very much flattened and concave, perhaps through pressure of the duct, and it is beginning to bend. It is marked here by several irregular longitudinal ridges and furrows.

In the 47.5 mm. stage still greater alterations of the original form of the post-valvular gut are evident (fig. 25). The contour is very irregular. Owing to the fact that the growth of this part of the intestine has exceeded that of the body cavity, the gut has bent in two places, each at right angles to the long axis of the body. A twisting of the gut likewise has occurred so that the duct of the digitiform gland extends laterally from it instead of dorso-laterally as in preceding stages (figs. 23 and 24). The dorso-ventral diameter of the gut in this region is small. The concavity of the gut into which the duct of the digitiform gland enters is very deep, the two sides nearly meeting, but they are separated by the duct. The longitudinal furrows and ridges are more numerous than in the previous stage and are much more marked. There are four large ridges, each bearing

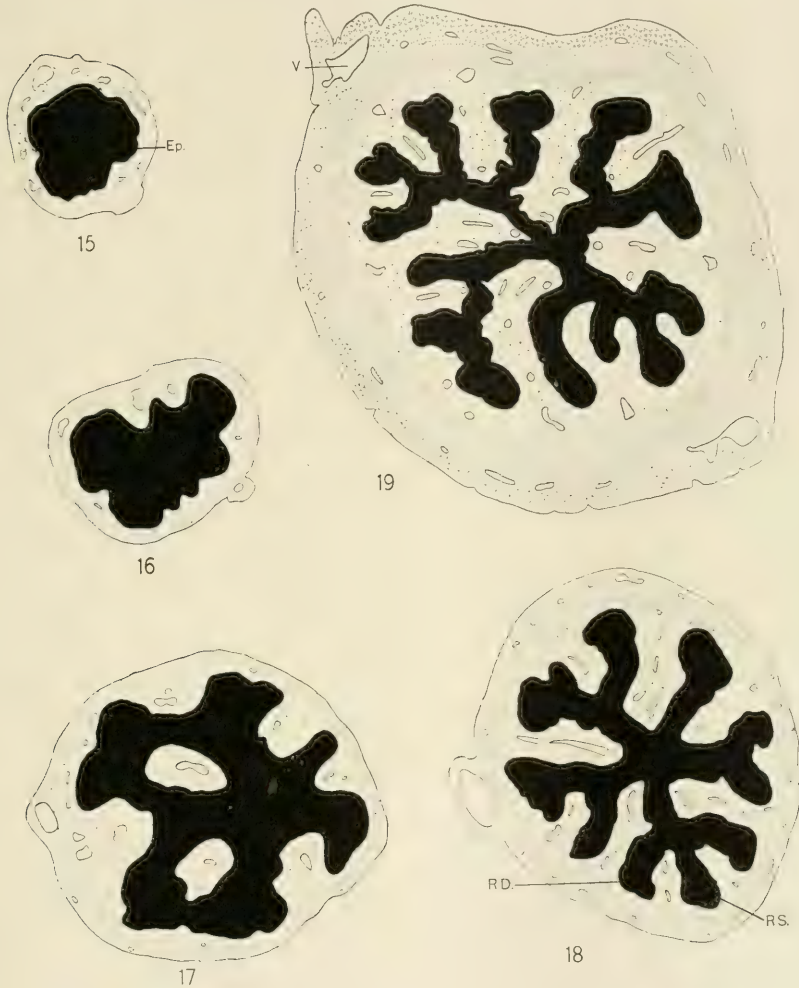
two or more smaller longitudinal ridges. The furrows between the ridges often extend under them and hence, in transverse section, appear as spaces in the epithelium filled with connective tissue (fig. 17). The spiral valve ends abruptly anterior to this region. The dorsal part of the cloaca into which the Wolffian ducts enter is marked off from the remainder of this segment of the gut.

The structures just described have become more highly differentiated in the 95 mm. stage (fig. 26). The angle of the two bends has changed from 90 degrees to less than 30, giving the intestine an 'S' shape. Three groups of ridges are present. One group of six ridges extends forward from the cloaca, gradually disappearing anteriorly. Of these, three are ventral and three dorsal. Of the latter, two are extensions of the lateral corners of the dorsal outpouching which receives the Wolffian ducts, and the other is midway between them. The middle group of ridges is that described in the preceding stage. The portion of the gut containing these ridges is greatly lengthened in the present stage and the digitiform duct is carried posteriorly. There are five principal ridges here, but each is subdivided at the periphery and appears to branch when seen in transverse sections (fig. 18). These ridges flatten posteriorly just above the digitiform duct, and anteriorly they disappear abruptly just behind the spiral valve (fig. 26). They are, in general, longitudinal in direction, but they twist slightly in the same direction as the turns of the spiral valve. A third group of numerous small ridges occurs on the exterior surface of the spiral valve, running nearly at right angles to the group just described.

The oldest embryo studied (200 mm.) was not modelled, but from study of sections and dissections the post valvular intestine appears to have straightened (fig. 9), while the solid ridges are still present on the external surface of the gut (fig. 19).

Occlusion of the post-valvular intestine

In the first three stages described (15, 19 and 22 mm., figs. 20, 21, and 22) the lumen of the gut is relatively large and uniform. In the wall of the gut (figs. 1 and 2) are five rows of cells.



Figs. 15, 16, 17, 18, and 19 $\times 50$. Sections through the middle of the occluded portion of the post-valvular intestine in *Acanthias* embryos.

Fig. 15 Embryo 28.1 mm. in length.

Fig. 16 Embryo 33.1 mm. in length.

Fig. 17 Embryo 47.5 mm. in length.

Fig. 18 Embryo 95 mm. in length.

Fig. 19 Embryo 200 mm. in length. *Ep.*, epithelium in solid black; *R.D.*, solid ridge which is continuous with the solid end of the duct of the digitiform gland (fig. 13); *R.S.*, solid ridge which is continuous with the intestine posterior to the occluded portion and which splits later to form the new lumen (fig. 13). Note the very small lumen in figure 15. The areas of connective tissue within the epithelium in figure 17 are continuous at a different level with that external to it.

In the 28.1 mm. stage the lumen of gut just posterior to the spiral valve narrows suddenly, but is still present. Its narrowest diameter, about half way between the spiral valve and the opening of the digitiform duct is two to four μ . Anterior and posterior to the portion of the gut with the narrow lumen the wall of the gut is composed of five rows of cylindrical cells with elongated nuclei. These gradually increase in number, and midway between the two limits just mentioned as many as twenty rows of nuclei are present, packed so closely together that they seem almost to touch one another. From the periphery toward the center of the gut, the first five or six rows of nuclei are elongated, resembling the usual type seen in the gut, but the nuclei lying internal to these are irregularly rounded. This indicates that the occlusion is brought about by proliferation of the cells lining the lumen.

In the 33.1 mm. embryo the gut is solid for about 0.2 mm. in the region midway between the spiral valve and the digitiform duct (figs. 24 and 16). At both ends of the solid portion the cavity of the gut widens abruptly to its normal diameter, but the transition in thickness of the epithelium is more gradual. Both anterior and posterior to the solid portion the wall contains from four to six rows of cylindrical cells with elongated nuclei. This layer is continuous through the solid portion, but, in addition, there is a large number of irregularly round cells around the center of the gut. The ridges on the external surface (fig. 24) appear to be produced by peripheral outpushing of the epithelium due to pressure applied at the center, and to be the result of central cell proliferation rather than of the growth of the peripheral epithelium or pressure of the surrounding mesenchyma. However, cell division at the periphery may aid in ridge formation. The mesenchyma covering the ridges appears to be under considerable pressure, as the cells are very flat as compared with those between the ridges. Smaller secondary ridges are found on the larger primary ones.

In the 47.5 mm. stage the gut is solid (figs. 25 and 17) for about 0.25 mm. just anterior to the point of entrance of the digitiform duct. The main ridges are broken still more into

smaller ones. In some adjacent places secondary ridges are still fused at the periphery, while separated toward the center. There thus appear to be, in transverse section, areas of connective tissue completely surrounded by epithelium (fig. 17). These connective tissue masses are not entirely cut off but are continuous with that surrounding the epithelium at some point anterior or posterior to the section in question.

In the 95 mm. stage the solid portion of the post-valvular intestine (fig. 18) extends anteriorly about 0.9 mm. from the entrance of the digitiform duct (fig. 26). The epithelium has been so completely divided and subdivided into ridges that the central part is less in diameter than the peripheral part of the ridges themselves (fig. 18). The ridges are narrow as compared with the preceding stage so that the surface of the epithelium has increased in much greater proportion than the volume. There are four principal ridges, each subdivided into two or three parts (fig. 18). In transverse sections the ridges appear as finger shaped processes. One of these ridges on the ventrolateral side is continuous with the duct of the digitiform gland and from the external form it is impossible to tell the point where the duct terminates (fig. 13). In a transverse section however (fig. 14) it is seen that the duct enters by a side opening into the dorsal side of the gut.

Around the periphery each ridge has from four to six rows of elongated nuclei, the long axes of which are at right angles to that of the ridge. The center of each ridge is made up of cells with irregularly rounded nuclei, although some are elongated, parallel with the long axis of the ridge. Later, when the lumen of the gut is re-established, the latter cells form the surface layer of epithelium. Small isolated lumina are seen in the solid epithelium toward either end of the solid region.

In the 200 mm. stage the solid portion of the post-valvular gut extends 2.2 mm. anterior to the ostium of the duct of the digitiform gland. The gut has straightened (fig. 9). There are four principal ridges upon the external surface, each subdivided into two to four parts. They are of greater diameter at the periphery than at the center of the gut. Histologically the

solid gut in this stage is much the same as that in the 95 mm. embryo. Each ridge shows four to six rows of elongated nuclei at the periphery, the two opposite halves being connected by central cells, irregular in form, with elongated nuclei lying at right angles to those of the peripheral cells. Toward the two ends of the solid region the small isolated lacunae in the epithelium are more numerous than in the preceding stage and in places some have united longitudinally, so that the solid epithelium of each ridge is beginning to split. This process must be completed shortly after this stage because a 200 mm. *Acanthias* is almost full term. The increase in the circumference of the lumen thus established, as may be noted by comparison of figures 15, 19, 20 and 21, is many times greater than the increase in the length of this portion of the gut, and thus possesses a surface area not much less than a segment of the spiral valve of equal length.

Summary of part 2

The post-valvular portion of the intestine in *Acanthias* in young stages is a simple tube. Various markings in the form of irregular longitudinal ridges and depressions soon appear on the external surface of the gut. The ridges increase rapidly in height. As the spiral valve twists, these ridges turn in the same direction.

The gut loses its circular outline (as seen in transverse sections) and becomes much flattened, especially in the region of the digitiform gland. Owing to a rapid longitudinal growth, the gut becomes bent in this region into an S form. A dorsal portion of the cloaca into which the Wolffian ducts early enter becomes marked off from the rest of this chamber to form a bladder.

The lumen of the gut in the region just anterior to the duct of the digitiform gland becomes occluded by a rapid proliferation of the cells in the center. These cells produce a centrifugal force which accounts largely for the ridge formation. The relatively thick solid plug of epithelium soon becomes almost completely divided into solid ridges which in turn are subdivided

extensively. These ridges later split through the center and there is thus formed an irregular lumen. The surface area of this segment of the gut thus produced is many times greater than that which existed before its occlusion.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

20, 21, 22, 23, and 24 Wax reconstruction of the post-valvular intestine (enteral) through the anlage of the digitiform gland of *Acanthias* embryos; models drawn from dorsal side.

20 Embryo 15 mm. in length; $\times 33$.

21 Embryo 19 mm. in length; $\times 33$.

22 Embryo 22.5 mm. in length; $\times 38$.

23 Embryo 28.1 mm. in length; $\times 38$.

24 Embryo 33.1 mm. in length; $\times 38$. In figure 24 only the proximal part of the gland is shown.

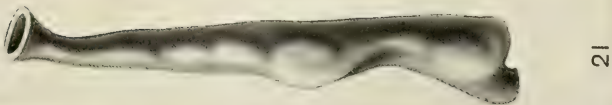
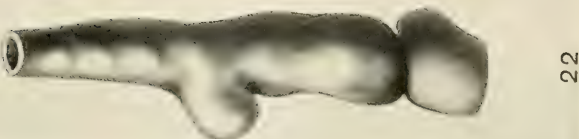
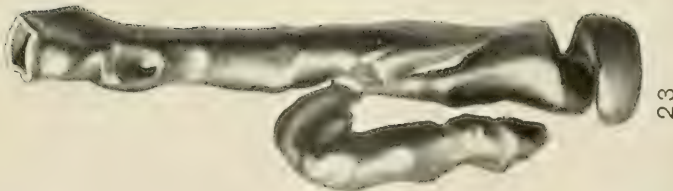
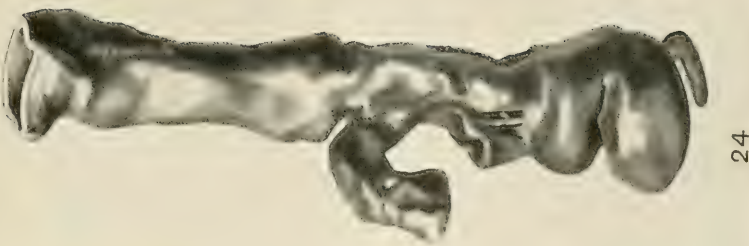


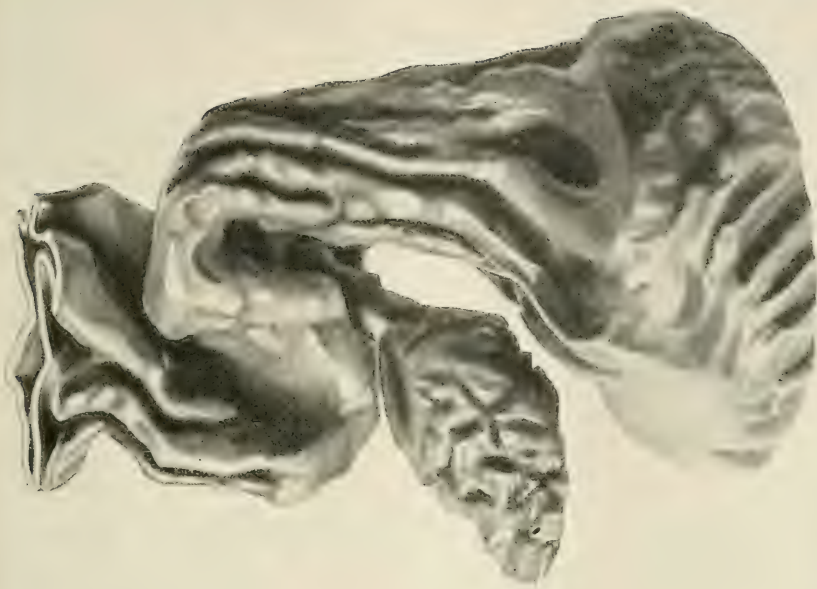
PLATE 2

EXPLANATION OF FIGURES

- 25 and 26 $\times 38$. Wax reconstruction of the post-valvular intestine (enteral) and proximal portion of the digitiform gland in an *Acanthias* embryo 47.5 mm. long; model drawn from the dorsal side.
- 25 Embryo 47.5 mm. in length.
- 26 Embryo 95 mm. in length.



25



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PLATE 3

EXPLANATION OF FIGURES

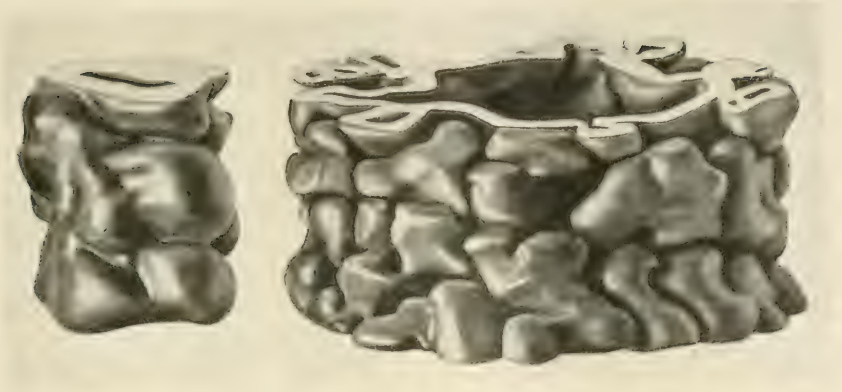
27, 28, 29, and 30 $\times 120$. Wax reconstruction of a segment taken from the middle of the digitiform gland, showing tubule formation in *Acanthias* embryos.

27 Embryo 28.1 mm. in length.

28 Embryo 33.1 mm. in length.

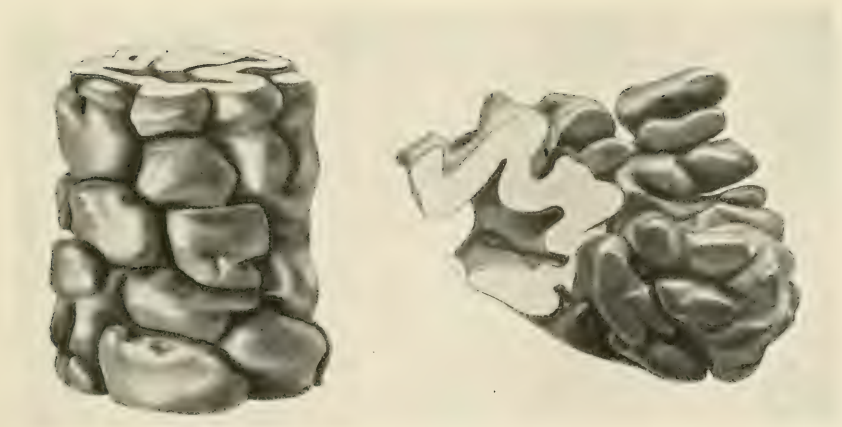
29 Embryo 47.5 mm. in length.

30 Embryo 95 mm. in length. In figure 30 only a small portion of the wall of the gland is shown.



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THE DEVELOPMENT OF THE SUPRAPERICARDIAL (POSTBRANCHIAL, ULTIMOBRANCHIAL)) BODY IN *SQUALUS ACANTHIAS*

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TWENTY-NINE FIGURES (TWO PLATES)

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INTRODUCTION

Much attention has been given in recent years to a study of the derivatives of the caudal portion of the pharynx in the higher vertebrates, particularly in mammals. Of these derivatives the ultimobranchial or postbranchial body has been perhaps the subject of the most discussion and has given rise to the most diverse opinions as to its significance and fate. From a review of the literature it is obvious that most investigators consider the ultimobranchial body homologous with the suprapericardial body of selachians. In this group little work has been done on the suprapericardial body since the original communication in 1885 of its discoverer, van Bemmelen. Owing to the absence of any complete description of the gland in a single form of the lower vertebrates, and because of the singular position it occupies among glands when classified according to form, it was thought that a detailed study of its development and structure in a single form (*Acanthias*) would be desirable. It is hoped

that such a study may help serve as a foundation upon which to base in the future a more exact consideration of the homology of this gland with similar structures in higher vertebrates.

The present paper is based upon a study of a series of sectioned embryos of *Squalus acanthias* from 19 to 95 mm. in length and of sections and dissections of the glands of a number of specimens of 'pups' (late fetuses), newborns and adults. Most of the sectioned material is from the Harvard Embryological Collection (H. E. C.) while some are from the embryological collection of Dr. R. E. Scammon (S. C.). The older specimens were obtained from the Harpswell Biological Laboratory.

I wish to thank Dr. Scammon for the loan of material and for his continued interest and many helpful suggestions in the progress of this work.

LITERATURE

The literature dealing with derivatives of the pharynx-wall and gill-pouches of fishes and amphibians is very extensive. Identical structures in the same species and homologous ones in closely related forms have been given different names and interpretations by various authors. The name 'suprapericardial body' applied on this paper is the one originally given to the structure by van Bemmelen ('85).

Van Bemmelen ('85), in a study of the rudimentary branchial clefts of elasmobranchs, describes the suprapericardial bodies as a pair of small epithelial masses just caudal to the last pair of branchial clefts. They extend through about half the thickness of the ventral pharynx-wall which immediately overlies the dorsal surface of the pericardial cavity. The bodies themselves begin to form in a stage when the last pair of clefts have just opened.

Originally the body is a simple evagination of the ventral pharyngeal epithelium. Soon, however, its blind end expands and forms a large ventral cyst. From the cyst small epithelial sprouts arise which later acquire lumina and become lengthened and coiled. Usually the small neck or connecting-piece becomes stunted and remains as an excretory channel, but in some

cases it completely disappears, leaving the bodies entirely isolated.

Histologically the bodies consist of small round follicles held together by scanty connective tissue. The walls of these follicles are formed of columnar or cubical epithelial cells which have large round nuclei. The cavities of the follicles are filled with a structureless mass containing an occasional nucleus. Van Bemmelen was not able to find the nerve-supply and blood-supply of the bodies, but thought that possibly their vascular branches were derived from the truncus arteriosus or the axillary artery.

Van Bemmelen described the bodies in a number of species of selachian embryos but was unable to find them in the mature *Heptanchus*. In *Chimaera* the body develops behind the sixth pouch, which completely degenerates. Often in *Acanthias*, *Scyllium*, *Pristiuris* and *Galeus* the body develops only on one side. In the *Raia*, *Aetobatis*, *Acanthias* and *Chimaera* the glands remain until maturity and attain a considerable size. In *Acanthias* he describes and figures the duct-opening of the bodies in the free surface of the pharyngeal mucous membrane on either side of the copula. Van Bemmelen considers the suprapericardial bodies as representing a rudimentary seventh pair of branchial pouches which fail to reach the ectoderm and have lost their original function. In support of this hypothesis he argues that they are degenerate structures and are homologous with epithelial derivatives of the spiracle and mouth in selachians, which he also considers as rudimentary clefts.

The same author ('89) continued the study of the suprapericardial body or its homologues, in the higher vertebrates. He found the body well developed in ganoids, amphibians and in *Lacerta*. In *Acipenser* it consists of follicles as in elasmobranchs. In one or the two examples of *Acipenser* studied the body was found only on the left side. In amphibians the body is always bilateral. It loses its connection with the pharynx very early and develops into a relatively large, thin-walled cyst lying free in the connective tissue. It remains as a simple cyst for a long period, but ultimately sends out diverticula which develop into

small independent follicles. He did not find the body in teleosts. In *Lacerta* the body is present only on the left side and lies much farther forward than it does in selachians and amphibians. Histologically it is very similar to that found in Amphibia. In birds and mammals van Bemmelen found great variation in the pharyngeal derivatives of the cervical region in the different forms. He homologizes with the suprapericardial body of selachians, the 'accessory thyreoids' of De Meuron in birds and mammals; the 'postbranchial bodies' of Maurer in amphibians; the 'Body Y' of Mall in birds; and the 'lateral thyreoids' of Born and His in mammals.

In a study of the pharyngeal derivatives in the higher forms, van Bemmelen urges great care in differentiating between those which arise from the pouches themselves and those which spring directly from the pharynx-wall. This is particularly true in mammals, where these derivatives, although very similar in structure, are to be distinguished by their mode and place of origin. He believes that the suprapericardial bodies in reptiles and their homologues in birds and mammals, as in the selachians and amphibians, represent a rudimentary pair of branchial pouches.

De Meuron ('86), in his studies on the development of the thymus and thyroid glands, gives a brief account of the suprapericardial body in selachians and amphibians. He homologizes the body in these forms with the accessory thyreoid of reptiles, birds, and mammals.

In *Acanthias vulgaris* (*Squalus acanthias*) De Meuron describes the suprapericardial body as a small diverticulum, arising from the ventral pharynx-wall, caudal to the last branchial cleft. Although the body may, primarily, arise symmetrically on the two sides, only the one on the left continues to develop. The right body rapidly atrophies and completely disappears. The left body, later in its development, separates from the pharynx, and divides into many small follicles.

In amphibians (*Rana* and *Bufo*) the bodies arise symmetrically on the two sides of the pharynx, caudal to the branchial clefts. Here the bodies always separate completely from the

pharynx and form small isolated cysts. Later, the surrounding connective tissue invades the organ, dividing it into two or three small lobules. In the adult these bodies come to lie so near the thyreoid that they are considered, by De Meuron, as accessory thyreoids.

In *Lacerta* a diverticulum of the ventral pharynx-wall arises behind the fourth gill-pouch on either side. The diverticulum on the right side atrophies very early, while that on the left persists. De Meuron calls this body the accessory thyreoid, and believes it is homologous with the suprapericardial body of selachians and amphibians.

In birds and mammals De Meuron describes symmetrical diverticula arising from the ventral extremities of the fourth pair of gill-pouches. He considers these diverticula as homologous with the accessory thyreoids of reptiles and the suprapericardial body of selachians and amphibians.

Maurer ('87), studying *Amphibia*, describes the suprapericardial bodies and calls them postbranchial bodies because of their position behind the last branchial pouch. In *Anura* they arise symmetrically from the ventral pharynx-wall behind the fifth branchial pouches. Later the bodies become separated from the pharynx and form, either a single large follicle or a complex of smaller ones. The follicles never develop colloid, but almost always, in some phase of their development, contain a serous secretion.

In urodeles the postbranchial body develops only on the left side. It arises as a solid epithelial bud which early acquires a small central lumen. This strand separates from the pharynx, becomes placed obliquely to the long axis of the pharynx and in some cases buds off a few smaller epithelial masses. Maurer did not find the postbranchial bodies in teleosts.

According to Maurer's description, the postbranchial bodies in amphibians in no way resemble the thyreoid and can not be called accessory thyreoids. They occasionally show a serous secretion but never develop colloid. Maurer believes that De Meuron described correctly the origin of the postbranchial bodies, but that he has confused their later development with

the epithelial derivatives of the pouches, which come to lie near the thyroid. He agrees with De Meuron, however, that the postbranchial bodies in Anura are homologous with the suprapericardial bodies of selachians. He leaves entirely undecided the question of the homology of the postbranchial bodies and the derivatives of the fourth pouches in birds and mammals. The unilateral development of the body in urodeles, and its rather marked variation in structure from that found in Anura, suggests to Maurer that possibly, in urodeles, the postbranchial body represents a remnant of the ductus oesophago-cutaneous of *Bdellostoma*.

Platt ('96), working with *Necturus*, described the suprapericardial bodies as small vesicles arising from the ventral pharyngeal wall, between the fourth and fifth branchial clefts on either side. They become separated from the pharynx but retain their primitive position and show no fusion with the thyroid. The position of the bodies in *Necturus*, according to Platt, opposes the view of van Bemmelen that they are to be regarded as rudimentary clefts and also the conception of Maurer that they are postbranchial structures.

In the tunicates Giard ('98) considers the epicardial tube homologous with the postbranchial body, an homology of the same nature as that of the endostyle with the median thyroid.

Greil ('05) opposes the view of Maurer that the suprapericardial body in amphibia is postbranchial. By reconstructions of the branchial region of the foregut in *Hyla* he found that the anlage of the sixth pouch appears on either side as a shallow outpouching of the lateral pharyngeal wall, caudal and slightly medial to the fifth pouch. The thickened epithelium of the ventral portion of this rudimentary sixth pouch develops into the ultimobranchial (suprapericardial, postbranchial) body. The bodies develop symmetrically on the two sides. The dorsal and medial segments of the sixth pouch form a cord of cells which runs caudalwards and unites with the gut. The ultimobranchial body is completely separated from the pharynx and contains a small central lumen. Greil believes that Maurer, overlooking the remaining segments of the sixth pouch, de-

scribed the fifth pouch as the last, and considered the derivatives of the rudimentary sixth pouch as postbranchial structures.

Greil also opposes the view of Maurer and De Meuron that the suprapericardial body in *Anura* develops as an outpouching of the pharyngeal wall. On the contrary he describes it as a solid thickening of the epithelium which develops a central lumen after its separation from the pharynx. He thinks that De Meuron has described the body in a fairly advanced stage of development—after the formation of a central lumen—and has mistaken this for its earliest anlage. Greil was unable to find the suprapericardial bodies in five specimens of *Bombinator* studied by him.

In selachians, Greil found the ultimobranchial body only on the left side. In *Acanthias* he describes the anlagen of the seventh pouches arising caudal and medial to the sixth pouches. The appearance of the seventh pouches is delayed by an elevation of the pharyngeal wall in the region of the sixth pouches. On the medial side of this elevation the ventral extremity of the seventh pouch on the left side develops into the ultimobranchial body.

Greil agrees with van Bemmelen that the ultimobranchial (suprapericardial) bodies represent rudimentary seventh pouches, especially their ventral extremities. He thinks that in *Chimaera*—in which the suprapericardial bodies are formed behind the sixth pouches (which degenerate without leaving any derivatives)—it is very probable that the loss of the sixth pouch is to be considered as a unique case, and a phenomenon to which the suprapericardial body has not yet adapted itself, as it has, for example, in the amphibians. In this latter group the formation of the suprapericardial body is assumed by the sixth pouch. In the higher groups, in which the reduction of the gill apparatus is more advanced and in which there are only four pouches, the suprapericardial body is formed from the last pouch.

Vialleton ('08), in his work on the visceral arches in vertebrates, describes briefly the suprapericardial bodies in *Torpedo*. In large embryos of this form (70 mm. in length) they consist of a short excretory canal closely surrounded by a few glandular

acini. The entire gland is small and lies very close to the pharyngeal wall to which it is attached by its excretory duct. There is no evidence of secretion in the gland at this stage.

TOPOGRAPHY AND MORPHOLOGY

The suprapericardial body or gland in *Acanthias* first appears in embryos of about 20 mm. in length. Although it is occasionally present later on both sides of the pharynx, its early appearance is always limited to the left side. The gland on the right side does not make its appearance until about the 33 mm. stage. The anlage of the gland appears as a simple evagination or outpouching of the epithelium of the ventral pharyngeal wall, medial and slightly caudal to the ventral extremity of the sixth gill-pouch (fig. 1).

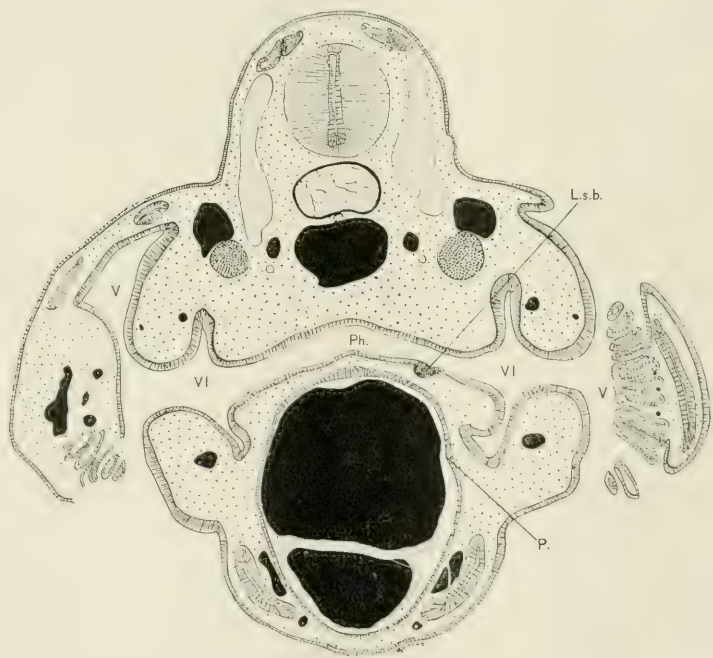


Fig. 1 Transverse section through the pharynx of an embryo 20.6 mm. long (H. E. C. 1494) at the level of the suprapericardial body. $\times 40$. The cavities of the heart and blood vessels are in solid black. Ph., pharynx; V, VI, fifth and sixth gill-pouches; P., pericardium; L.s.b., left suprapericardial body.

Figure 2 is from a wax reconstruction of the pharynx of an embryo 20.6 mm. long (H. E. C. 1494), showing the position of the gland at the time of its appearance. This embryo is No. 28a of Scammon's Normal-plate and corresponds approximately with Balfour's stage N. The pharynx is rather flat and arches dorso-ventrally. Its cranial extremity is broad and relatively large but rapidly tapers caudally into the flat and narrow oesophagus. All of the gill-pouches are formed. They have a rather wide origin from the lateral margins of the pharynx, and project outward at almost right angles to the main axis of the embryo. Their dorsal extremities are bent slightly caudalward and are much more pronounced than the ventral ones, which are small, narrow and crowded close to the wall of the pharynx. All of the pouches with the exception of the first are open along their ventral and lateral margins. The thyreoid is represented by a single median elongated mass at about the level of the second pair of gill-pouches. It is in close proximity to the ventral wall of the pharynx from which it has but recently lost its connection. The anlagen of the thymus have not made their appearance.

The suprapericardial body at this stage is found only on the left side. It arises from the pharynx slightly caudal to the origin of the ventral extremity of the sixth gill-pouch. It is located just medial to a continuation of the line joining the origins of the ventral extremities of the pouches. The epithelium forming the gland is slightly thickened and evaginated into the mesenchyma to about half the distance to the pericardium which surrounds the atrium (fig. 1).

In slightly older embryos (24.7 mm. H. E. C. 1492) the suprapericardial body has about the same relative position as in the specimen just described. At this stage also the body is found only on the left side. The small plate of epithelium has become converted into a digitiform tubule which reaches almost to the pericardium. The tubule is slightly constricted at the point of its connection with the pharyngeal epithelium. It contains a very small lumen which communicates directly with the cavity of the pharynx.

Figure 21 is from a wax reconstruction of the left supra-pericardial body of an embryo 28 mm. in length (H. E. C. 1357, stage 30 of Scammon's Normal-plate). Here the digitiform tubule of the preceding stage has become converted into a single rounded vesicle with a connecting-stalk, which has a

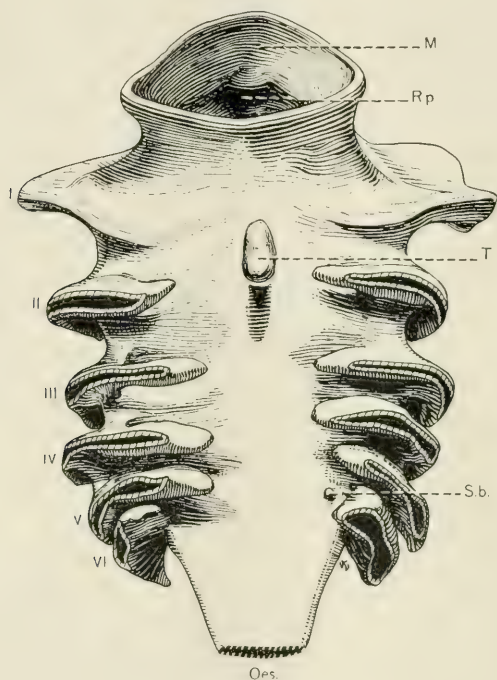


Fig. 2 Ventral view of a wax reconstruction of the pharynx of an *Acanthias* embryo 20.6 mm. long (H. E. C. 1494). $\times 24$. The gill-pouches have been cut off close to their origin from the pharynx. *M.*, mouth; *R.p.*, Rathke's pouch; *T.*, thyreoid; *I* to *VI*, first to sixth gill-pouches; *S.b.*, left supra-pericardial body; *Oes.*, oesophagus.

broad attachment to the vesicle but is constricted at its connection with the pharyngeal wall. It contains a narrow lumen which communicates above with the pharynx and below with the saccular cavity of the vesicle. The stalk is attached to the caudal portion of the cyst, giving the gland a cranialward directed development which later becomes more marked.

The earliest appearance of the suprapericardial body on the right side is found in embryos of about 33 mm. in length (S. C. 8 and H. E. C. 186). It has about the same corresponding position as the body on the left side. The relation of the glands to the pharynx and its derivatives at this stage is shown in figure 3 which is from a wax reconstruction of the pharynx of an embryo 33.1 mm. long (S. C. 8). The arching of the pharynx present in the younger embryos (fig. 2) has almost entirely disappeared. The cavity of the pharynx has become somewhat narrowed in its dorso-ventral diameter because of the rapid growth of the underlying heart. The gill-pouches have a broad attachment to the lateral regions of the pharynx. Each succeeding pair of pouches, the second to sixth inclusive, arises from the pharynx a little lateral to the pair preceding. This condition is more apparent than real, because the ventral extremities of the more caudal pouches are pushed ventrally and laterally by the growth of the heart. The expanded distal portions of the pouches are arched caudally so as to imbricate the succeeding ones. The thyreoid is a shield-shaped mass of tissue located in the median line about on a level with the origin of the second pouch. Remnants of its early connection with the pharyngeal wall are found in a thin broad pouch just below the mouth. The thymus is represented by knob-shaped epithelial thickenings on the dorsal extremities of the last five pair of pouches.

The suprapericardial bodies are medial and slightly caudal to the ventral extremities of the sixth gill-pouches. They have assumed a position somewhat more medial than that in the younger embryos, lying about half way between the sixth pouch and the median line. The right suprapericardial body is much smaller and more rudimentary than the left. It consists of two slender villous-like cords of epithelium connected to the pharyngeal epithelium. There is no lumen present in either of the cords. Van Bemmelen describes a similar condition in *Acanthias* where he found the gland consisting of three small ducts opening into the pharynx.

The left suprapericardial body in the 33.1 mm. embryo (S. C. 8) is roughly triangular in shape. The connecting-stalk is at-

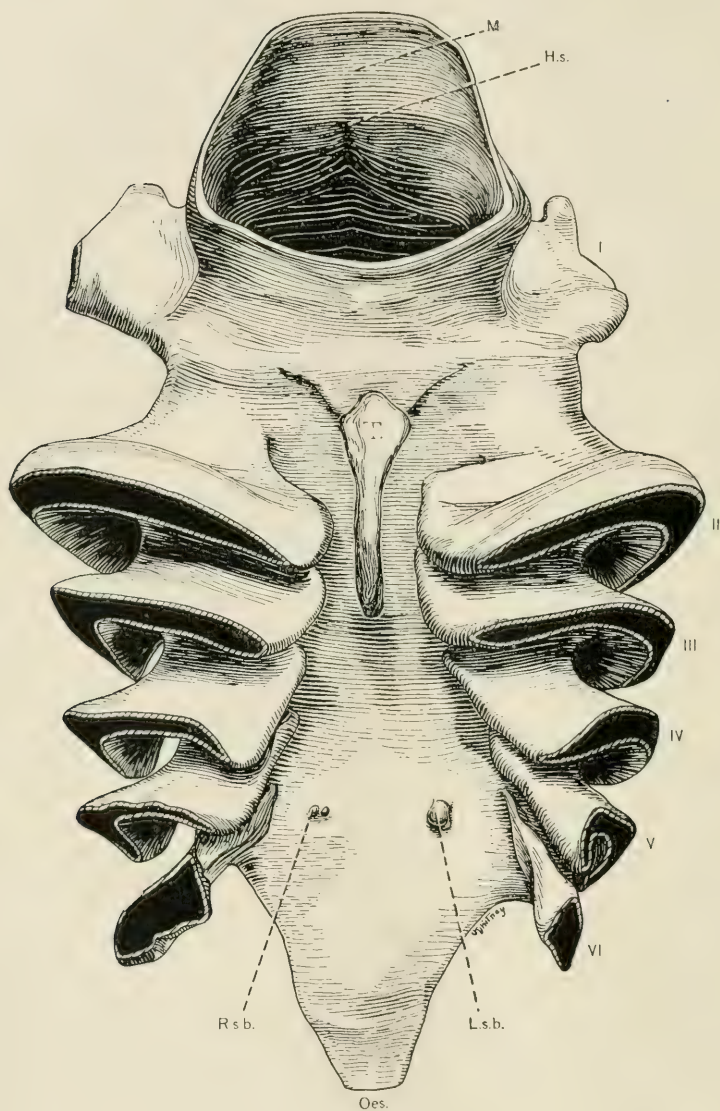


Fig. 3 Ventral view of a wax reconstruction of the pharynx of an embryo 33.1 mm. long (S. C. 8). $\times 24$. The gill-pouches have been partly cut off and their cavities represented in black. *M.*, mouth; *H.s.*, hypophyseal stalk; *I* to *VI*, first to sixth gill-pouches; *T.*, thyroid; *R.s.b.*, right suprapericardial body; *L.s.b.*, left suprapericardial body; *Oes.*, oesophagus.

tached to the caudal and lateral portion of the ventral mass giving the gland a direction of growth which is distinctly medial and forward. The lumen of the stalk communicates with that of the vesicle and with the pharynx. The attachment of the stalk to the pharyngeal epithelium is very much constricted and is indicated by a small depression in the ventral pharyngeal wall.

From the 33 to the 37 mm. stage the suprapericardial body shows great advance in development. In the series of five embryos studied of this stage (S. C. 25; S. C. 10; S. C. 9; H. E. C. 186 and H. E. C. 363) the bodies were found only on the left side. They have about the same position as in the preceding embryo. The form of the gland is shown in figure 22 from a wax reconstruction of the left gland of a 37 mm. embryo (H. E. C. 363). The ventral portion consists of an elongated thin-walled vesicle running parallel to the long axis of the pharynx. It is constricted at its middle and terminates posteriorly in two rounded knobs. A small bud is arising on its medial surface. The connecting-stalk is long and angular and is attached to the dorsal, caudal portion of the vesicle. A small bud which arises from the stalk is directed cranialward. The lumen of the connecting-stalk is small and irregular and does not communicate with the pharyngeal cavity nor with the cavity of the vesicle. As is shown in the above description, two important features appear in the gland at this stage (36-37 mm.). They are the beginning formation of new tubules by budding and a disappearance of the earlier communication found between the ventral portion of the gland and the pharynx.

At 47.3 mm. (S. C. 11 and others) the suprapericardial body is again found on both sides. The right gland is very small and is situated at about the level of the cranial extremity of the left gland (fig. 4). The caudal portion of the pharynx at this stage has become arched from side to side because of the dorsal bulging of the heart. This change in the contour of the pharyngeal wall has caused the suprapericardial body to assume an oblique position, being directed medially and ventrally from its attachment to the pharynx. Immediately below the sixth pouches the pharynx becomes abruptly constricted transversely to form the oesophagus.

The right suprapericardial body in the 47.3 mm. embryo (S. C. 11) is shown in figure 23. It consists of an expanded vesicle attached to the pharyngeal wall by a solid connecting-stalk. Its external form is very similar to that found in the left gland in the 28 mm. embryo (fig. 21). The left body is shown in figure 24. At this stage of development, the gland has become divided into three distinct parts. The dorsal part (A)

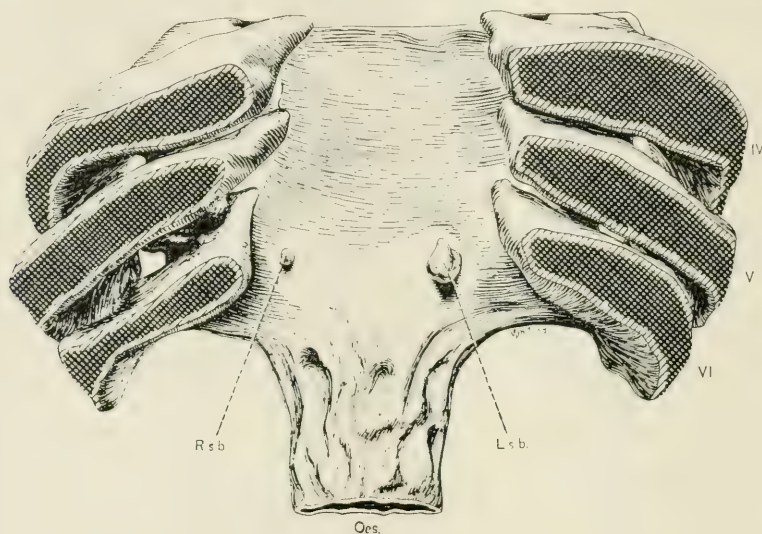


Fig. 4 Ventral view of a wax reconstruction of the caudal half of the pharynx of an embryo 47.3 mm. long (S. C. 11). $\times 18$. IV to VI, fourth to sixth gill-pouches; Oes., oesophagus; R.s.b.-L.s.b., right and left suprapericardial bodies.

consists of a single isolated tubule broadly attached to the pharyngeal epithelium. It contains a small lumen which communicates with the pharyngeal cavity. The medial portion of the gland (B) is a longitudinally directed tubule which is bifurcated at its cranial extremity. Its upper or dorsal surface is in contact with the tubule (A) but does not fuse with it. Its lower surface is in contact with one of the tubules of the ventral portion of the gland. Their epithelial walls are shown fused in only one section of $12\ \mu$. The ventral portion of the gland is

formed of a large tubule (*C*) and a broad flattened vesicle (*D*). These two masses are united at their caudal extremities and have a common cavity. Both *C* and *D* give rise to small tubules and epithelial buds. A comparison of figures 22 and 24, shows that in older embryos the connecting-stalk has become broken into segments which correspond to *A*, *B* and *C*. The ventral vesicle has steadily increased in size and now gives rise to a few small tubules. It still retains its connection with the stalk-element (*C*). In a slightly older embryo, 50 mm. long, (H. E. C. 444) these changes have become somewhat more marked. The growth of the gland has been chiefly in its cranio-caudal axis. The ventral vesicle has become very much elongated. The stalk-element is represented by many small tubules and buds, one of the most dorsal of which unites with the pharyngeal epithelium.

The position of the gland at 60 mm. (H. E. C. 427) is shown in figure 5. It is attached to the wall of the pharynx by a rather long tubule and also by a small cord of epithelial cells situated just caudal to the tubule. It has the same general form as in the 50 mm. embryo but has many more newly-formed tubules, some of which are completely isolated and lie in the mesenchyma of the pharyngo-pericardial wall.

The oblique position of the gland and its shift to a more medial position, which were first noticed in the 33.1-47.3 mm. embryos, have become more pronounced at 95 mm. (H. E. C. 1882). These features are shown in figure 6, from a wax model of the caudal portion of the pharynx at this stage. There is a rather sharp ventral outpouching of the wall of the pharynx just lateral to the suprapericardial body on either side. It begins just above the attachment of the gland and runs obliquely outward and backward parallel to the ventral diverticulum of the sixth pouch. On the dorsal wall of the pharynx, and forming its caudal extremity on either side, are two outpouchings very similar to those described above. These outgrowths, both dorsal and ventral, were first found in an embryo 80 mm. in length (S. C. 60). They are parallel to the corresponding extremities of the

sixth pouch and are located exactly in the place where one would expect the anlage of the seventh pouch to appear.

The suprapericardial bodies are attached to the medial wall of the ventral outpouchings of the pharynx. The right gland is very small and is at about the level of the base of the left one.



Fig. 5 Sagittal section through the pharynx of an embryo 60 mm. long (H. E. C. 427). $\times 30$. II to IV, second to fourth gill-pouches; *Ph.*, pharynx; *At.* atrium; *Vt.* ventricle; *C.c.*, ceratobranchial cartilage; *L.s.b.*, left suprapericardial body; *M.cb.*, musculus coracobranchialis.

It is a single vesicle, partly embedded in the pharyngeal epithelium. Its lumen does not communicate with the cavity of the pharynx (fig. 7). The left gland, shown in figures 7 and 26, is connected to the pharyngeal epithelium by three small tubules. The connecting-stalk has developed into a branching mass of tubules, a few of which apparently anastomose. The distal portion of the gland forms an elongated, obliquely placed vesicle, directed cranially. It gives rise to a few secondary tubules along its margins and dorsal surface. It is fused with some of the tubules immediately dorsal to it, but has an independent lumen.

In embryos of the 'pup' stage (20 to 22 cm. in length) and in the newborn and adult the gland is easily found by dissection under the binocular microscope. Although many specimens were examined, particularly of the 'pups,' the right gland was never found in these older stages. Upon removing the dorsal pharyngeal wall the position of the left gland is indicated by a small pit in the floor of the pharynx just lateral to the cardiobranchial (basibranchial) cartilage. Figure 29 is a cleared dissection of the head of a 'pup' 21 cm. long, showing the gland in situ. The ventral muscles, heart, and a portion of the pectoral girdle have been removed, as well as the dorsal musculature of the head and dorsal wall of the pharynx. The body or main portion of the gland lies between the cardiobranchial cartilage and the fifth arch of the left side. It is partly covered at its base by the ceratobranchial cartilage and the caudal portion of the coracobranchial muscle.

The caudal half of this gland was reconstructed and is shown in figure 27. The dorsal part of the connecting-stalk is broadly attached to the pharyngeal epithelium and has a deep blind pit in its center. Its ventral portion is solid and fuses with some of the adjacent tubules. When compared with the gland in the 95 mm. embryo it is found that the entire gland has greatly increased in size. The large ventral mass of tubules and vesicles has become relatively farther removed from the pharyngeal epithelium. The connecting-stalk is single and much longer, and appears to be a direct outpouching or diverticulum from

the epithelial wall of the pharynx. The ventral portion of the gland, especially in its cranial half, is formed of a large dilated vesicle, broken up in its caudal portion into several smaller vesicles and tubules. The large mass of tubules between the connecting-stalk and the ventral vesicle is very irregular and complicated. Some of the tubules are completely isolated, but many

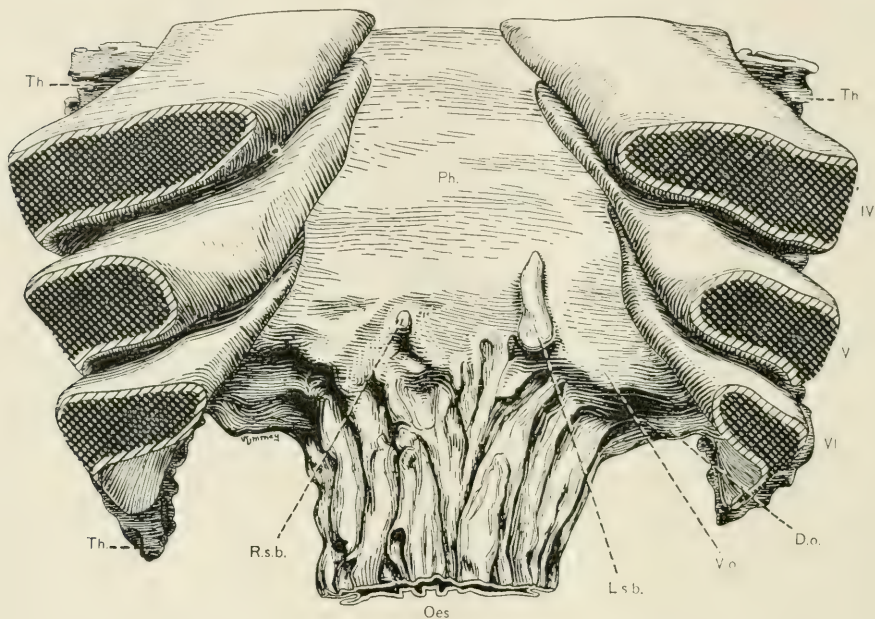


Fig. 6 Ventral view of a wax reconstruction of the caudal half of the pharynx of an embryo 95 mm. long (H. E. C. 1882). $\times 12$. *Ph.*, pharynx; *IV* to *VI*, fourth to sixth gill-pouches; *Th.*, thymus; *D.o.*, dorsal outpouching; *V.o.*, ventral outpouching; *R.s.b.*-*L.s.b.*, right and left suprapericardial bodies; *Oes.*, oesophagus.

of them are fused over small areas of their walls, but contain independent lumina. Apparently true anastomoses are found in a few places.

Figure 28 shows the tubules of the gland of a newborn fish (23.5 cm. long) which was cleared in oil containing enough stain to bring out the gland tubules and blood vessels distinctly. The caudal half of the gland rests upon the coracobranchial muscle

and receives a sheath from its fascia. The form of the gland at this time is very similar to that of the 'pup.' The pharyngeal diverticulum, located at about the middle of the gland, is solid and is much shorter than that of the 'pup.' The whole gland at

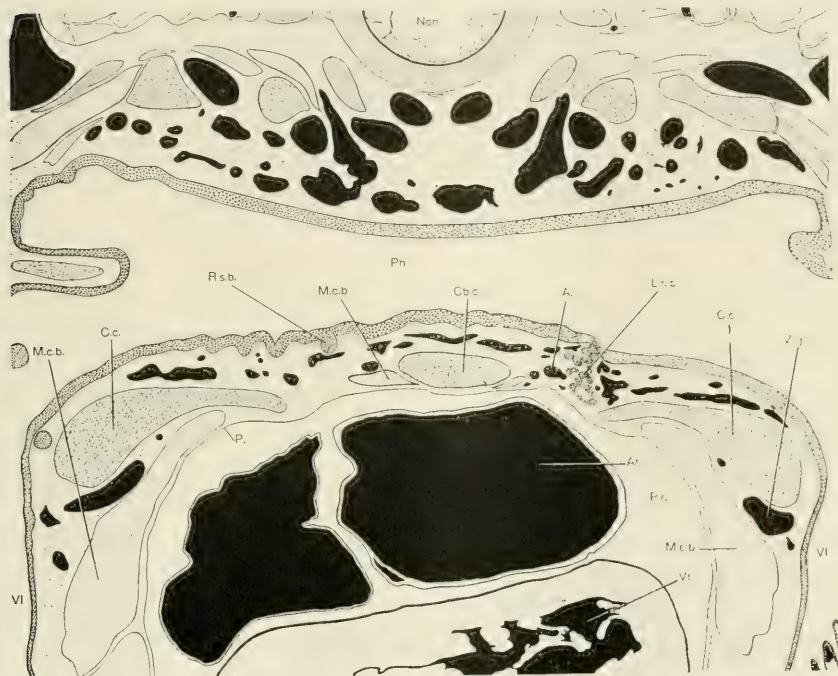


Fig. 7 Transverse section through the pharynx of a 95 mm. embryo (H. E. C. 1882) at the level of the suprapericardial bodies. $\times 24$. *Nch.*, notochord; *Ph.*, pharynx; *M.cb.*, musculus coracobranchialis; *C.c.*, ceratobranchial cartilage; *P.*, pericardium; *Cb.c.*, cardiobranchial cartilage; *A.*, artery; *V.i.j.*, inferior jugular vein; *R.s.b.*-*L.s.b.*, right and left suprapericardial bodies; *VI*, is placed in the ventral diverticula of the sixth gill-pouches; *At.*, atrium; *P.c.*, pericardial cavity; *Vt.*, ventricle.

this stage appears to be the result of repeated budding and branching of the tubules of the earlier stages. The greatest proliferation has occurred in its caudal half between the connecting-stalk and the large tubules which represent the termination of the ventral vesicle.

In a newborn fish (27 cm. in length) the gland was found only on the left side. It is very similar in structure to that described in the 'pup.' Many of the tubules have become dilated into large vesicles which communicate with each other and with the smaller tubules. The cranial portion of the gland has also developed into numerous small branching tubules and isolated vesicles. Two solid downgrowths of the pharyngeal epithelium are present, one being in the caudal and the other in the cranial portion of the gland. The most cranial one is the larger and is fused with the epithelium of one of the adjacent tubules. The downgrowth in the caudal portion of the gland is small and narrow and is fused with the epithelium of one of the gland tubules in only one section of 10 micra.

From the newborn to the adult the gland grows considerably in size. Although there is some formation of new tubules, the growth is due chiefly to an increase in size of the individual elements. The majority of the smaller tubules present in the earlier stages have, in the adult, become converted into large thin-walled vesicles which are elongated or irregular in shape, and communicate with adjacent vesicles by constricted necks. A number of the vesicles, especially in the extremities of the gland, are completely isolated. The gland lies embedded in the connective tissue of the pharyngo-pericardial wall, between the ceratobranchial cartilage laterally and the cardiobranchial cartilage and coracobranchial muscle medially. Some of the most caudal vesicles are flattened in their dorso-ventral axis as a result of being squeezed into the interval between the cartilage and muscle.

A large diverticulum or invagination of the pharyngeal epithelium, similar to that described in the 'pup', is present in the adult gland. It begins at about the level of the middle of the gland and extends almost to its caudal extremity. It is directed obliquely downward and toward the median line and is entirely independent of the gland vesicles adjacent to it. It contains a large saccular cavity, which opens directly into the cavity of the pharynx. A portion of the adult suprapericardial gland has a true duct opening into the pharynx. It consists of a small

mass of tubules and vesicles located in the extreme dorsal region, just cranial to the pharyngeal diverticulum. The duct is short and at its ventral extremity divides into two branches, one running caudally to end in a small tubule which terminates blindly, and the other running cranially to open into a rather large vesicle. This vesicle and tubule, when followed through the sections, are found to be entirely independent of the remaining portion of the gland. The duct and its connections has been modelled and are shown in figure 25. The lumen of the duct is small and opens directly into the cavity of the pharynx at the bottom of a deep longitudinal groove in the pharyngeal epithelium.

HISTOLOGY AND HISTOGENESIS

At the time of its appearance the suprapericardial body is a simple saccular evagination of the pharyngeal epithelium. The pharynx at this time is lined by a single layer of cells, cubical in shape near the median line, but gradually becoming columnar at the attachment of the gill-pouches. The suprapericardial body arises just lateral to the region where this transition occurs (fig. 1). The cells forming the anlage of the gland are decidedly columnar in shape (fig. 8). Their nuclei are located in their basal portions; are elongated oval in outline, and show a definite chromatic reticulum. The bulk of the chromatin is near the nuclear membrane, but a few masses are found scattered throughout the nucleus. The nuclei of the cells forming the medial wall of the outpouching at its union with the pharynx are very much elongated and closely crowded together, due to a slight twisting of the mass toward the median line. A definite *membrana propria* separates the epithelial cells from the underlying mesenchyma.

At 24.7 mm. (H. E. C. 1492) the saccular outpouching has become converted into an elongated tubule extending from the pharyngeal epithelium to the parietal pericardium. The cavity of the tubule communicates widely with the pharynx and is lined by a single layer of low columnar cells. The tubule, although short, is very tortuous, and, at the points of flexure, the cells are very much elongated and deeply stained. A little later in

the development (28 to 33 mm.) the blind ventral extremity of the tubule expands into a small vesicle which becomes bent in a medial and cranial direction. This bending of the gland and its extension medially and cranially, which becomes very prominent in the older embryos, is probably due to the resistance offered by the dorsal pericardial wall. The cells lining the pharynx at this stage are cubical or low columnar and have broadly oval nuclei (fig. 9). In the region of the pharynx lateral to the gland, the epithelium possesses a double row of rounded nuclei. Although not shown in the figure, the cavity of the connecting-stalk communicates with the pharynx and the dilated vesicle, both of which are lined by columnar cells with regular oval nuclei.

The gland remains in this condition, becoming somewhat larger and more expanded, up to about the 36–37 mm. stage, when it is somewhat variable in its finer structure. It usually shows a solid connection with the pharynx and its ventral portion becomes more or less separated from the connecting-stalk. Both the vesicle and the tortuous connecting-stalk show the beginning formation of new tubules. Often the larger expanded vesicle has already become divided up into two or three smaller independent ones, which also show the formation of new tubules.

Since the further growth of the gland from this time up until about the newborn stage, consists chiefly of the multiplication and branching of the gland elements, i.e., the tubules—this process will be considered in detail. The bulk of the small newly formed tubules always occurs between the ventral vesicle and the epithelial wall of the pharynx, showing that in all prob-

Fig. 8 Transverse section through the left suprapericardial body and pharyngo-pericardial wall of an embryo 20.6 mm. long (H. E. C. 1494); iron haematoxylin. $\times 525$. *P.*, pericardium.

Fig. 9 Transverse section through the left suprapericardial body of an embryo 33.1 mm. long (S. C. 8); alum haematoxylin. $\times 400$. *C.s.*, connecting-stalk; *V.*, vesicle; *P.*, pericardium.

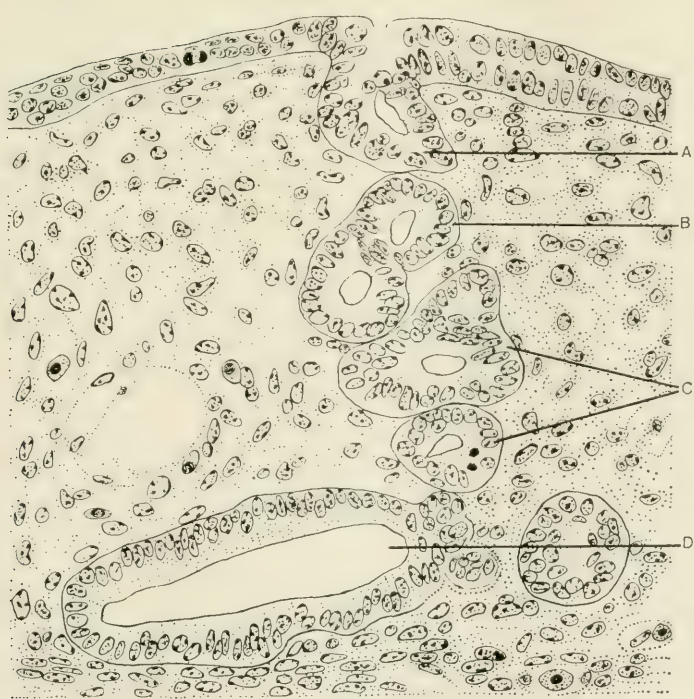
Fig. 10 Transverse section through the cranial portion of the left suprapericardial body of an embryo 47.3 mm. long (S. C. 11); alum haematoxylin. $\times 285$. *A.*, *B.*, *C.*, remnants of early connecting-stalk; *D.*, ventral cyst, showing two small buds on its right border.



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9



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Figs. 11 to 15 Sections of gland tubules illustrating the process of tubule formation.

Fig. 11 Thickening of epithelium; from an embryo 47.3 mm. long (S. C. 11); alum hematoxylin. $\times 450$.

Fig. 12 Beginning formation of outpouching and extension of lumen; from an embryo 95 mm. long (H. E. C. 1882); alum cochineal. $\times 450$.

Fig. 13 Pinching off of new tubule by ingrowth of cells from the sides; from an embryo 47.3 mm. long (S. C. 11); alum hematoxylin. $\times 450$.

Fig. 14a Further ingrowth of cells from the sides, showing the nuclei arranged tangentially with the lumina; from an embryo 95 mm. long (H. E. C. 1882); alum cochineal. $\times 450$.

ability the power of growth is greatest in the elements which represent the connecting-stalk. In this region, in any embryo, from 36 to 230 mm. long, the process of budding or new tubule formation can readily be studied.

The formation of new tubules and the branching of tubules will be discussed together, as both processes, so far as can be determined, are analogous, up to a certain stage in their development. At the site of a future tubule or branch a local proliferation of cells occurs. This is followed by a direct extension of the lumen of the parent tubule into this bud. The process may end here, resulting in simple branching, or the new sprout may become constricted at its base and later become cut off, forming a new isolated vesicle. The first step in the process—a thickening in the epithelium of the parent tubule—is very transitory, being followed almost immediately by an extension of the lumen into the new cell mass. Usually the newly formed cells are not numerous and form only a very small bud which remains surrounded by the membrana propria (fig. 11). Their nuclei are broadly oval or round and are closely crowded together. The cytoplasm of the cells is small in amount and stains deeply. Cell membranes could not be distinguished. These small buds of cells are quite numerous and are found both on the sides and at the extremities of the tubules.

The extension of the lumen into the cell mass is probably due to a more rapid growth of the wall of the tubule at this point. When once a definite outpouching is established, the cells become radially arranged around the cavity (fig. 12). The attachment of the outpouching to the wall of the parent tubule shows a slight constriction into which the surrounding mesenchymal cells push. The epithelial cells forming the attachment are narrow and elongated and closely crowded together on one side, due to a slight twisting of the outpouching toward that side. A

Fig. 14b Same section as 14a, but taken at a lower focus in which the tubules are separate, showing the changes in the form and arrangement of the nuclei. $\times 450$.

Fig. 15 Another specimen of the last stage of tubule formation showing more clearly the rotation of the nuclei as the tubules separate; from an embryo 47.3 mm. long (S. C. 11); alum hematoxylin. $\times 450$.

very similar condition, it will be recalled, was found in the anlage of the gland.

The newly formed outgrowth may remain connected and grow en masse with the parent follicle, forming a branching tubule, or may become pinched off, forming a new vesicle. The former condition is more common when the buds arise from the extremities of the parent tubule. It is not unusual to find in these cases three, or sometimes four, small sprouts arising from the extremity of a single tubule (fig. 24, *c*, and fig. 26). Secondary branching or dichotomous division of tubules does not occur. A newly formed branch does not give rise to a new gland element until it has become pinched off as a vesicle.

The process of separation of the tubules is by simple constriction of its connection with the parent tubule. This process can be found in all its stages, especially in the older embryos where the tubules are more numerous. The connection of the new branch, which presumably persists for some time, becomes gradually larger as the tubule grows. At the outset of constriction, the wall of the branch at its connection gradually grows in across the lumen of the junction (fig. 13). Usually this ingrowth occurs on all sides, but if the branch is bent upon the tubule it may occur only on one side. The constriction or ingrowth of the wall is always accompanied by the surrounding mesenchymal cells.

The nuclei of the cells of the ingrowing walls have, at first, a radial position with respect to the lumen. As the constriction proceeds and the cells from the opposite walls fuse, the nuclei become very much elongated and assume a position in which their long axes are tangential to the lumen. Figures 13 and 14*a*, show that this change in the position of the nuclei is not due to a rotation of either the nuclei or the cells, but results simply from the inward migration of the cells. When the new tubule or follicle becomes completely separated from the parent tubule the nuclei are again found placed radially with respect to the lumina (fig. 14*b*). Whether this change in the direction of the axes of the nuclei is due to a rotation of these bodies through an arc of 90 degrees, or whether it results from a change

in the shape of the nucleus, could not be determined with certainty. Figure 14*b*, is a view of the same two tubules shown in figure 14*a*, but is taken at a lower focus in which the tubules are separate. The nuclei of the adjacent, newly separated walls, especially on the right side, are oval or triangular, giving an appearance which suggests that the change in the axes of the nuclei is due to a change in their shape. In figure 15, however, in which the tubules are nearly completely separated, the nuclei are elongated or broadly oval and show varying degrees of obliquity which one would expect to find if the nuclei rotated during the process of separation. As the majority of the branching and budding tubules show changes very similar to these described for figure 15, it would appear that this process was most probable and that the variations in shape of the nuclei seen in figure 14*b*, are due to the pressure of the adjacent tubule. The tubules at first are separated only by a *membrana propria*, but later the mesenchyma and blood vessels intervene.

The cells in the wall of the fused tubules are too numerous and the cell walls too indistinct to follow the change in position of the cell itself, but in all probability the rotation of the axis of the nucleus represents a rotation of the entire cell. If this be true this process would be comparable to a reversal of the changes which have been described by Scammon ('15) in the anastomoses of hepatic tubules in selachians.

Figure 10 is from a section through the cranial portion of the gland modelled in figure 24, showing the termination of the elements *B*, *C* and *D* in small tubules. The dorsal tubule, *A*, is a direct continuation of the epithelium of the pharynx. Two small solid buds arise from the right border of the ventral vesicle. Caudally the large vesicle tapers into a tubule which has become enclosed by the developing fibers of the coracobranchial muscle.

The epithelial cells of the tubules vary in shape, from low columnar in the smaller to a high columnar in the larger tubules and vesicle. The nuclei are either broadly oval or elongated and occupy the basal portion of the cell. The pharyngeal epithelium at this stage (47.3 mm.) is definitely stratified, showing two or three rows of oval nuclei. Numerous mitotic figures

occur both in the gland cells and in the epithelium of the pharynx. The mesenchyma surrounding the gland is still loose and scanty except in the region between the vesicle and the pericardium, where it is very dense and contains many nuclei, arranged parallel to the surface of the pericardium.

From the 47.3 mm. embryo up to the 'pup' stage (200 to 210 mm.) the increase in the number of the gland elements is very great. In the 95 mm. embryo (H. E. C. 1882) the tubules have become so numerous that the mesenchyma has been pushed aside, leaving their epithelial walls in direct contact (figs. 7 and 26). The walls of three of the most dorsal tubules are fused with the epithelium of the pharynx, but a distinct lumen through the pharyngeal epithelium could not be traced. There is no pit or indentation on the surface of the pharynx that would lead one to suspect a communication.

The process of secretion in the gland begins at a period prior to the 'pup' stage (20-21 cm. in length). The exact time could not be determined from the series of embryos at my disposal. In the 95 mm. embryo (H. E. C. 1882) there are a few goblet cells in the pharyngeal epithelium, but there is no evidence of secretion in the gland. In a 'pup' 21 cm. in length many of the cells, both of the gland tubules and of the pharyngeal epithelium, are actively secreting mucus. The smaller tubules are lined by a single layer of narrow columnar cells with elongated oval or rod-shaped nuclei in the basal portions. The larger tubules have, in addition to the columnar cells, a layer of smaller flattened cells lying next to the membrana propria. The mucous secreting cells are rather numerous and are found in both layers. The mucus may be located in either the free portion or the basal portion of the cell. In some instances the secretion encircles the nucleus and distends both the cuticula and the membrana propria. The lumina of the tubules contain masses of secretion of variable size in which are a few desquamated epithelial cells and lymphocytes.

As will be seen from the model shown in figure 27, the gland is connected with the pharynx by a single elongated diverticulum, extending obliquely from left to right, which is a direct

extension downward of the pharyngeal epithelium. It is composed of a thick stratified squamous epithelium and contains in its center a small blind pit, continuous dorsally with the cavity of the pharynx. The caudal, ventral portion of the diverticulum fuses with the epithelium of one of the small dorsal tubules of the gland, but there is no communication between their cavities. The stratified epithelium of the diverticulum, as well as that lining the pharynx, contains numerous mucous goblet cells scattered through its entire thickness (fig. 16).

The mesenchyma of the pharyngo-pericardial wall of the earlier embryos has now developed into a fibrous connective tissue. Underlying the pharyngeal epithelium the fibers are collected into dense, compact bundles which project up under the epithelium, forming broad square-topped papillae. Surrounding the gland tubules, also, the fibers are relatively dense as compared to the coarse-meshed intervening stratum.

In the newborn fish, although the gland is actively secreting mucus, typical cells distended with mucus are not numerous. The tubules and vesicles still vary greatly in size. Some of the smallest elements of the gland are cords of cells without a lumen, in which the distended mucous cells are most frequently found. The tubules and vesicles are lined with a single layer of cubical or columnar cells. The lumina are either partially or completely filled with secretion, containing a few necrotic cells. Mitotic figures are found in most of the tubules, but are especially numerous in the larger vesicles. The epithelial downgrowths of the pharynx, mentioned in the earlier description of the newborn animal, are composed of stratified squamous epithelium. They both contain mucous goblet cells and are very similar to the diverticulum described in the 'pup,' except they do not contain a true lumen which communicates with the cavity of the pharynx. The larger downgrowth, located in the cranial portion of the gland does contain a small blind cavity, but it is formed by the fusion of several large globules of mucus (fig. 17).

The connective tissue in the caudal portion of the gland forms a distinct sheath surrounding the tubules, which becomes con-

tinuous laterally with the perichondrium of the ceratobranchial cartilage, and medially with the fascia of the coracobranchial muscle. In the cranial part of the gland the sheath thins out and gives way to loose vascular connective tissue. There are no connective tissue septa present, consequently the gland is not divided into lobes or lobules.

The large vesicles of the adult gland are lined by a layer of tall and narrow columnar cells, many of which are moderately distended with secretion. The nuclei are oval or rod-shaped and stain very intensely. In some parts of the gland the vesicles are closely crowded together and their epithelial walls are flattened through pressure. Two small tubules in the dorsal part of the gland, lying on either side of the tubule which connects with the true duct described above, are lined throughout a part of their course by a thin layer of stratified squamous epithelium. One of the large closed vesicles in the cranial extremity of the gland is also lined by stratified epithelium which has many goblet cells. Another large vesicle has a small rounded extremity which contains so many mucous cells that, when viewed in cross-section, it resembles very closely an ordinary mucous alveolus. The pharyngeal diverticulum mentioned above is composed of stratified squamous epithelium directly continuous with that lining the pharynx. The diverticulum, like the epithelium of the pharynx, contains mucous goblet cells in all layers from the membrana propria to the surface (fig. 18). The true duct connecting a part of the gland with the pharynx, is formed by a single layer of columnar cells which become continuous with the basal row of cells of the pharyngeal epithelium.

ANGIOGENESIS

The earliest vessels associated with the suprapericardial body are a few small venules which extend into the pharyngo-pericardial wall from the inferior jugular vein. In embryos, 33 to 36 mm. long, these vessels consist of irregular mesenchymal spaces lined by a layer of developing endothelial cells. At 47.3 mm. these irregular spaces or vessels have become more uniform in calibre and are filled with red blood corpuscles. They have

increased both in size and number and in some places are in close proximity to the walls of the tubules. In the pharyngo-pericardial wall just caudal to the gland, the vessels form a coarse-meshed plexus which communicates laterally with the inferior jugular vein and caudally with the common cardinal vein.

From the 47.3 mm. to the 95 mm. stage the veins surrounding the growing tubules increase greatly in number and form a close-meshed plexus which completely envelops the gland except on its ventral surface (fig. 7). The vessels of the plexus have very thin walls, and in most cases they are separated from the gland tubules only by the *membrana propria*.

A number of small arterial twigs ramify in the mesenchyma between the tubules. They are branches from a larger bilateral trunk in the pharyngo-pericardial wall, just lateral to the cardio-branchial cartilage and in close proximity to the gland. It arises cranially from the sinus formed by the median hypo-branchial artery and terminates posteriorly by dividing into the pharyngeal and gastric arteries.

In the 'pup' stage (21 cm. long) the veins surrounding the gland tubules are larger, but relatively less numerous than those found at 95 mm. Although the mesenchyma of the earlier stages has at this time become converted into a fine-fibered connective tissue, the walls of the veins are still very thin. In many places, however, the walls of the vessels are invaginated by small nodules of lymphocytes in the center of which is found an arteriole.

Accompanying the veins, and in most cases actually embedded in their walls are numerous clefts or channels which I have interpreted as lymphatics. These channels, many of which lie in close proximity to the walls of the gland vesicles, communicate freely with the vessels previously described as veins (fig. 18). The arrangement of the vessels here is very similar to that described by Ferguson ('11) in the thyroid of elasmobranchs. Ferguson, using the terminology of Favaro, calls the veins '*venae lymphaticae*,' and the lymphatics '*vasa lymphatica*.' The content of these vessels in the thyroid, Ferguson believes, depends upon the blood pressure, which is determined by the

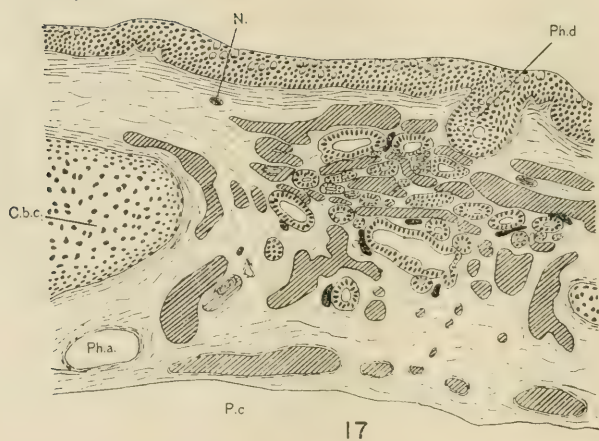
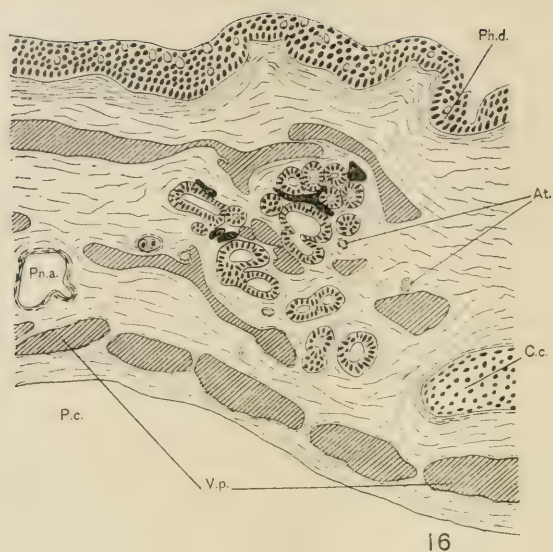


Fig. 16 Transverse section through the suprapericardial body of a 'pup' 21 cm. long; alum cochineal. $\times 75$. *Ph.d.*, cranial extremity of the pharyngeal diverticulum; *Ph.a.*, pharyngeal artery; *C.c.*, ceratobranchial cartilage; *At.*, arterioles; *V.p.*, subpericardial venous plexus; The vasa lymphatica are represented in solid black; the venae lymphaticae by parallel diagonal lines. *P.c.*, pericardial cavity.

Fig. 17 Transverse section through the suprapericardial body of a newborn 23.5 cm. long; iron hematoxylin. $\times 50$. *Ph.d.*, pharyngeal downgrowth; *C.b.c.*, cardiobranchial cartilage; *Ph.a.*, pharyngeal artery; *N.*, nerve; *P.c.*, pericardial cavity; vasa lymphatica in solid black; venae lymphaticae in parallel diagonal lines.

contraction and expansion of the muscles of the mouth and pharynx. If the pressure be low the vessels carry lymph and if high they carry blood. Such a primitive arrangement of the circulation is common in fishes, and, although I have not had



Fig. 18 Transverse section of the suprapericardial body of an adult, passing through the pharyngeal diverticulum, *Ph.d.*; *M.cb.*, musculus coracobranchialis; *P.c.*, pericardial cavity; *C.c.*, ceratobranchial cartilage; *Ph.a.*, pharyngeal artery; vasa lymphatics in solid black; venae lymphaticae in parallel diagonal lines; alum hematoxylin. $\times 35$.

opportunity to study the circulation by injection of fresh specimens, I feel certain this condition is present in the suprapericardial body.

In the newborn fish the large venae lymphaticae are very numerous and form a rich plexus which so completely envelopes

the gland, that, in some of the sections, the vesicles appear to be suspended in the vascular network (fig. 17). The vessels surrounding the gland drain ventrally into a plexus of larger vessels lying just under the pericardium. This subpericardial plexus drains laterally into the inferior jugular vein. I have seen no muscle-whorls in any of the specimens examined.

No striking differences are to be found in the circulation of the adult from that described for the 'pup' and newborn. The *venae lymphaticae* are larger and in some places their walls contain lymphoid nodules of considerable size (fig. 18).

DISCUSSION

The original conception of van Bemmelen ('85) that the suprapericardial body in selachians represents a rudimentary branchial pouch is probably correct. This conception is shared also by Greil ('05) who describes in selachians a true anlage of a seventh pouch. I was unable to find the early anlage of the seventh pouch as described by him. In the embryos of much older stages, 80–95 mm. in length (S. C. 60 and H. E. C. 1882), there is a distinct outpouching of the pharyngeal wall, both dorsal and ventral, on either side, and exactly in the place where one would expect a seventh pair of pouches to arise (fig. 6). The suprapericardial body arises from the medial wall of the ventral outpouching. In the younger stages, particularly at the time of appearance of the gland or shortly before, no such outpouchings are to be found. In one embryo of 28 mm. (S. C. 6) the left suprapericardial body forms a single diverticulum which extends ventrally almost to the pericardium (fig. 19). It is widely open to the cavity of the pharynx and is lined by a single layer of tall columnar cells, very similar to those of the gill-pouches. This gland resembles very closely in its appearance a developing pouch. There is no corresponding structure on the right side nor any indication of a dorsal outpouching above it. From the position of the gland—medial and slightly caudal to the sixth pouch—and from its mode of development as an elongated diverticulum from the pharynx which resembles (very strikingly in one embryo, S. C. 6) a developing pouch, I believe

we are justified in assuming that in *Acanthias* the suprapericardial body represents the ventral extremity of a rudimentary seventh gill-pouch. In many of the specimens which I have examined, the gland in its early stages appeared like a rudimentary pouch which had become stunted in its progress of growth by abutting against the underlying pericardial wall.

The failure of the gland to appear on the right side in many of the specimens is difficult to explain. From my material it

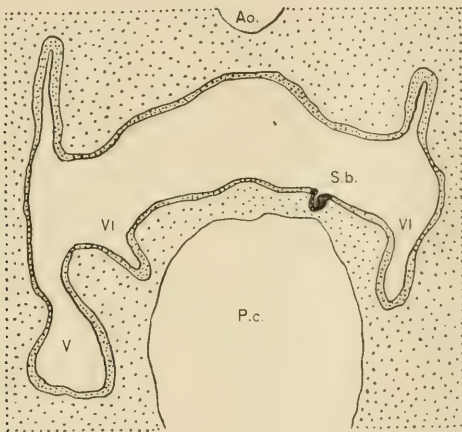


Fig. 19 Semidiagrammatic section through the pharynx of an embryo 28 mm. long (S. C. 6) at the level of the left suprapericardial body. $\times 40$. *Ao.*, aorta; *V, VI*, fifth and sixth gill-pouches; *P.c.*, pericardial cavity; *s.b.*, suprapericardial body.

appears to be the rule rather than the exception that the right body fails to appear. Van Bemmelen ('85) found that often in *Acanthias*, *Pristiuris* and *Galeus* the body appears only on the left side. He figures a dissection of the 'pup' stage showing the openings of the gland on either side of the cardiobranchial cartilage. Although numerous specimens of the 'pup' were examined, I found the gland only on the left side (fig. 29). All of these specimens however were not sectioned and possibly in some cases the right gland was present, but too small to be seen with the binocular microscope. De Meuron ('86) says that in

Acanthias the body may, primarily, arise symmetrically on the two sides but that the right gland rapidly atrophies and disappears. Greil ('05) in his work on selachians found the body present only on the left side. From these observations and from the fact that the right gland, when present, appears relatively late (33 mm. or later) and is always represented as a single small vesicle, it is very probable that it entirely degenerates in the course of development.

If the mode of development of the suprapericardial body be carefully analyzed, it will be found that it differs both from the ordinary type of branching gland (tubulo-alveolar) and the typical closed-follicular ductless gland. The ordinary type of gland in selachians develops as a direct outpouching of the epithelium. This outgrowth is usually preceded by a thickening of the epithelium to form a gland bud, as has been described in the gastric glands by Peterson ('08), in the pancreas and liver (Scammon '14; '15) and in the digitiform gland (Hoskins '15). The blind extremity of this outpouching gives rise to the tubular or saccular end pieces while its connecting-stalk forms the excretory canal. The end pieces may become secondarily removed from the main excretory duct, but they always remain in communication with it (fig. 20, A, 1, 2, 3). The ductless glands, typical examples of which Laguesse ('10) has described as glands with closed follicles, develop as an outpouching of the epithelium which almost immediately becomes solid. This solid anlage becomes broken up into cell-cords or plates (Norris '16) which acquire lumina and develop into follicles or vesicles, the most of which are completely closed (thyreoid, fig. 20, C, 1, 2, 3). The connecting-stalk or excretory canal usually entirely disappears in most forms early in the development. Occasionally, however, vestiges of the original duct are found. In the thyreoid of man these vestiges form accessory thyreoid masses or develop into cysts or tumors. Goodey ('10) has described vestiges of the original thyreoid evagination in *Chlamydoselachus anguineus*, *Scyllium catulus* and *Scyllium canicula*, in the form of a small duct or a mass of accessory thyreoid follicles which occupies a foramen in the basibranchial cartilage immediately above the

thyreoid. The duct opens directly into the oral cavity, but posteriorly it is closed and is united to the thyreoid only by connective tissue.

The suprapericardial body in its development and structure is intermediate in type between the ordinary branching gland and the follicular ductless gland. It arises as an evagination of the epithelium which very soon develops into a solid connecting-stalk and a blind expanded portion. A part of the connect-



Fig. 20 Diagrams showing three corresponding stages in the development of (A) a typical branching gland in selachians; (B) the suprapericardial body; and (C) a closed-follicular gland (thyreoid).

ing-stalk forms a large diverticulum which opens into the pharynx, and is entirely separate from the rest of the gland. The remaining part of the connecting-stalk and the blind expanded portion of the gland develop into large irregular vesicles, the most of which intercommunicate. A small part of the gland near its point of origin becomes secondarily connected to the pharynx by a true duct (fig. 20, B, 1, 2, 3).

The glandular epithelium of the portion of the gland having a duct is identical in structure and appearance with that of the

independent vesicles, and both have a mucous secretion. The secretion of this portion is discharged into the cavity of the pharynx, while that of the independent portion of the gland is collected in the vesicles. Although distending some of the vesicles considerably, the secretion retains its granular, reticular appearance and does not become 'colloidal.' From the fact that some of the vesicles are completely isolated and that the others, although intercommunicating, are still lacking in any structure which could serve as an excretory canal, it may be inferred that their secretion is taken up by the vasa lymphatica, although no evidence of this process was observed.

Since this paper is devoted solely to a study of the gland in one form, the question of the homology of the suprapericardial body with similar glands in other forms will be discussed only briefly. Van Bemmelen did not find the suprapericardial bodies in teleosts, but Supino ('07) describes postbranchial bodies in *Leptocephalus* lying between the pharynx and pericardial wall, and Giacomini ('09) found them not only in *Leptocephalus* but also in adult *Anguilla*. These bodies are easily differentiated from the thyreoid and are undoubtedly homologous with the suprapericardial bodies of selachians. In *Stomias boa*, Nusbaum-Hilarowicz ('16) describes a small paired gland lying in the connective tissue, ventral to the pharynx. Caudally the two glands are united. Each portion of the gland is formed of a number of tortuous tubules, surrounded by epithelial strands and capillaries. The tubules are lined by a cylindrical, ciliated epithelium, and contain a granular secretion. This structure, which Nusbaum-Hilarowicz considers a heretofore undescribed ductless gland, corresponds very closely to the postbranchial body found in other teleosts and can be homologized with the suprapericardial body of selachians.

De Meuron ('86) describes small epithelial bodies arising behind the last gill-pouch in amphibians, reptiles, birds and mammals which he calls accessory thyreoids and which he considers as homologues of the suprapericardial body of selachians. From De Meuron's description, as Maurer ('87) has already pointed out, it is very probable that De Meuron has confused

the postbranchial body with the epithelial derivatives (epithelial bodies III and IV) of the gill-pouches. Maurer ('87) describes in *Amphibia* a derivative of the pharynx posterior to the last gill-pouch which he calls the postbranchial body and considers this as the homologue of the suprapericardial body. Greil ('05) found that the postbranchial body of Maurer developed, in *amphibia* as in *selachians*, from the ventral extremity of a rudimentary sixth pouch and believes for this reason that the body is ultimobranchial. In the higher vertebrates an homology with the suprapericardial body is difficult to make because of the rudimentary development of the pharyngeal pouches. In his later communication, van Bemmelen believes that the 'Body Y' of Mall ('87) in birds and the 'lateral thyroids' of Born ('83) and His ('80) in mammals are homologous with the suprapericardial body. Later writers,—Rabl ('07, birds), Grosser ('10, man), and particularly Verdun ('98, mammals) describe a diverticulum arising from the pharynx near the connection of the fourth pouch. This body unites with the thyreoid and forms a small cyst which, in some forms, contains colloid (Hermann and Verdun, '99). Getzowa ('11), studying human thyroids which had become atrophic, believes that the ultimobranchial body does not form thyreoid tissue, but remains cystic or entirely degenerates. This view is also held by Kingsbury ('14). As to the significance of the ultimobranchial body in man Kingsbury says:

Continued growth produces the blind pocket of pharyngeal entoderm termed the ultimobranchial body. It may be described as produced by a continuation of the growth process in the pharyngeal entoderm, which as part of the differential growth of the region has formed the successive branchial pockets. In its development it would from this point of view be linked thus with the branchial region. It could hardly represent in any morphological sense a rudimentary fifth pouch, since it is or appears to be already present in the two embryos in which a fifth pouch is shown. Nor does it seem to me that there is any better reason for describing it as an appendage of a fifth pouch. Into it, in later development would undoubtedly go the cells which actually took part in a fifth ento-ectodermal contact, or might have done so had it been developed.

He also believes that the ultimobranchial body in man does not represent a vestigial ancestral gland, and that, with the possible exception of the ultimobranchial body of birds, no gland has been found in the forms below mammals with which the ultimobranchial body may be homologized. If we admit the contention of Kingsbury that the ultimobranchial body in man is branchiogenetic in its origin, then it would appear without justifiable doubt that this structure could be homologized with similar branchiogenetic organs in lower forms, including the suprapericardial body of selachians. The distinction of whether the gland in question develops from a degenerating pouch or simply from the epithelium which has the potentiality of forming a rudimentary pouch seems to me to be unnecessary. The more recent work on the ultimobranchial body by Badertscher ('16) in the pig tends to show that these bodies do not degenerate entirely, but contribute to the formation of the structural elements in the thyroid gland.

SUMMARY

1. The suprapericardial body of gland in *Acanthias* appears in embryos of about 20 mm. in length. It arises as an outpouching of the epithelium of the ventral pharyngeal wall, medial and slightly caudal to the sixth gill-pouch. In its position and mode of development the gland corresponds to the ventral extremity of a rudimentary seventh pouch.

2. The epithelial outpouching forming the anlage of the gland rapidly develops into a solid connecting-stalk and a blind, expanded ventral portion. The dorsal part of the connecting-stalk remains as a diverticulum from the pharyngeal epithelium. The remaining part of the connecting-stalk and the expanded ventral portion of the gland become converted into large, distended vesicles, most of which intercommunicate, but some of which are completely isolated. A part of the gland in the adult may secondarily become connected with the pharynx by a true duct. In its development and structure, therefore, the suprapericardial body represents a type of gland intermediate in

structure between the ordinary branching gland and the closed follicular or typical ductless gland.

3. The early development of the gland is always limited to the left side. The right gland is inconstant in its development and does not appear until about the 33 mm. stage. When present, the right gland has the form of a single cyst or solid cord of cells which degenerate completely in the course of development.

4. The vesicles of the adult gland are derived from branching tubules which develop from the expanded ventral portion of the gland and the connecting-stalk. The process of formation of the new tubules can, for convenience of description, be divided into three stages:

a. A small, but definite, localized proliferation of cells at the site of the future tubule.

b. An extension of the lumen of the parent tubule into the new cell mass, the cells of which rotate so as to become placed radially with respect to the new lumen. The newly-formed outpouching may remain connected and grow en masse with the parent tubule forming a branching tubule; or

c. It may become constricted off by ingrowth of the epithelial walls from one or both sides. The nuclei of the cells of the ingrowth become placed tangentially with respect to the circumference of the lumen, but when the tubule is completely separated they rotate through an arc of 90° to assume a position radial to the new lumen.

5. Throughout the later development of the gland and in the adult, the tubules and vesicles are closely surrounded by a 'rich' plexus of thin-walled veins which drain laterally into the inferior jugular trunk. Accompanying the veins and imbedded in some cases directly in their wall is a network of vessels which I have interpreted as lymphatics. The lymphatics and veins communicate freely and are analogous to the 'venae lymphaticae' and 'vasa lymphatica' of the thyroid of selachians, as described by Ferguson.

6. The vesicles are lined by a single layer of narrow columnar cells which are actively secreting mucus. The nuclei are elon-

gated oval or rod-shaped and located in the basal portion of the cells. In the adult the secretion of a part of the gland is conveyed to the pharynx by a small duct. The secretion of the greater part of the gland is, however, stored in the vesicles and may taken up by the vasa lymphatica.

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PLATE 1

EXPLANATION OF FIGURES

21 Left lateral view of a wax reconstruction of the left suprapericardial body of an embryo 28 mm. long (H. E. C. 1357). $\times 125$. The attached pharyngeal epithelium is shown in part.

22 Left lateral view of a wax reconstruction of the left suprapericardial body of an embryo 37 mm. in length (H. E. C. 363). $\times 125$.

23 Left lateral (medial) view of a wax reconstruction of the right suprapericardial body of an embryo 47.3 mm. long (S. C. 11). $\times 125$.

24 Left lateral view of a wax reconstruction of the left suprapericardial body of an embryo 47.3 mm. long (S. C. 11). $\times 125$. The elements *A*, *B*, and *C*, represent the early connecting-stalk; the element *D* the ventral vesicle.

25 Left lateral view of a wax reconstruction of a portion of the left suprapericardial body of an adult showing its connection with the pharynx through a true duct. $\times 30$.

26 Left lateral view of a wax reconstruction of the left suprapericardial body of an embryo 95 mm. long (H. E. C. 1882). $\times 125$.

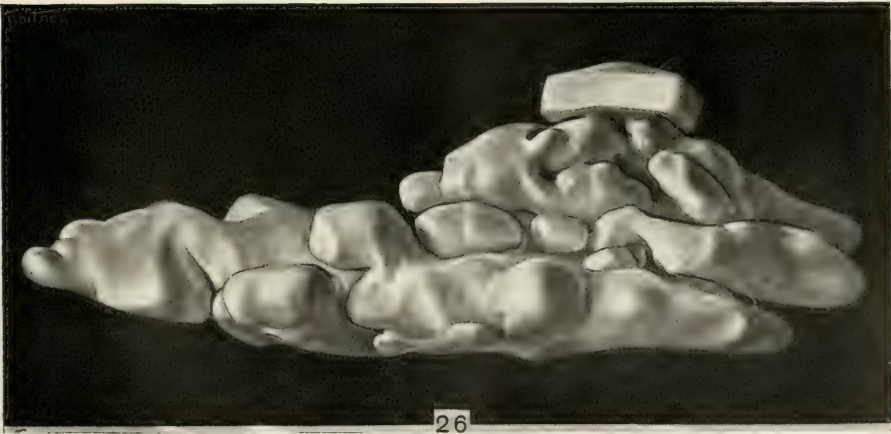
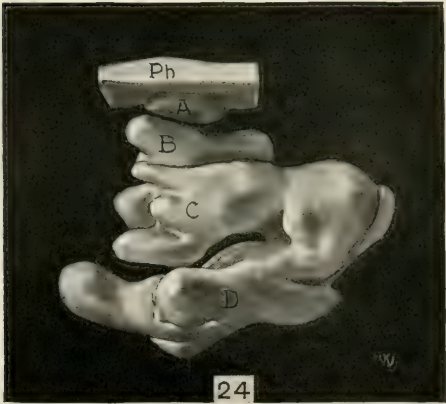
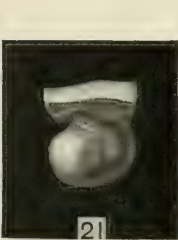


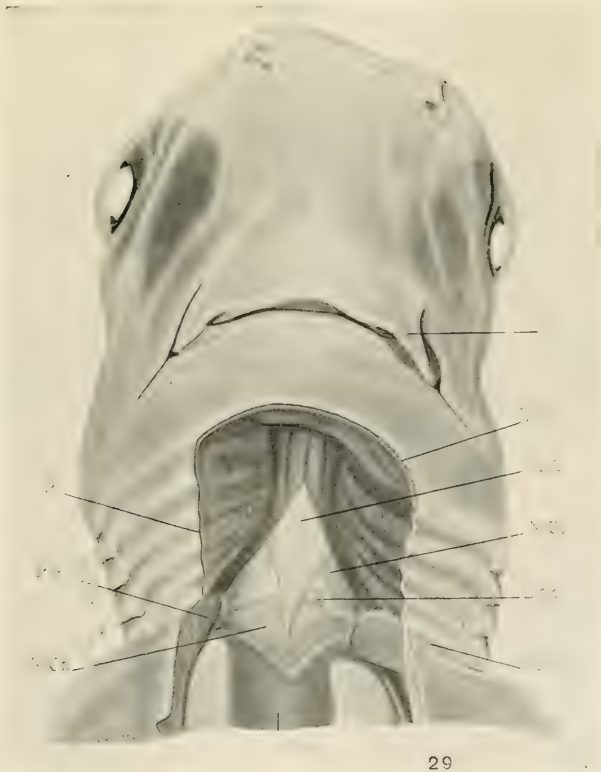
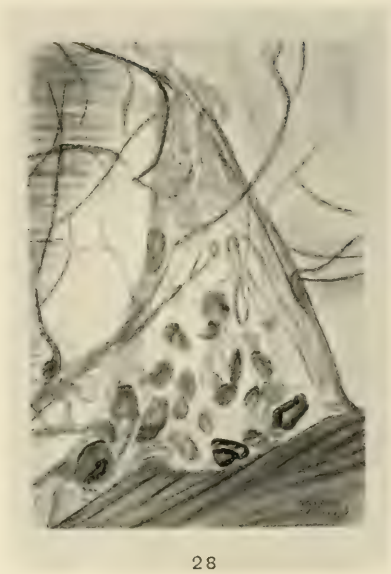
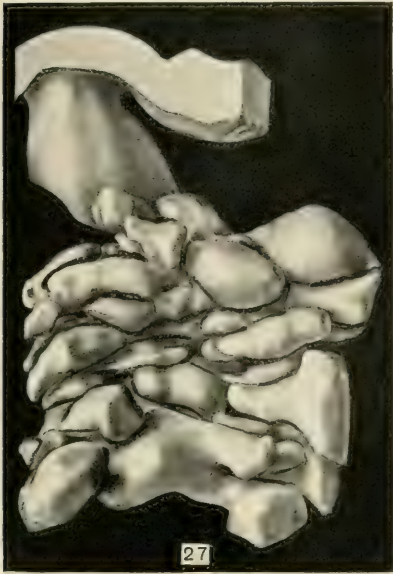
PLATE 2

EXPLANATION OF FIGURES

27 Left lateral view of a wax reconstruction of the caudal portion of the left suprapericardial body of a 'pup' 21 cm. long. $\times 90$.

28 Ventral view of a cleared specimen of the left suprapericardial body of a newborn *Acanthias* 23.5 cm. long, showing the gland tubules and blood vessels; eosin. $\times 30$.

29 Ventral view of a cleared specimen of the head and pharynx of a 'pup' 21 cm. long, showing the suprapericardial body in situ. $\times 2\frac{1}{2}$. *M.*, mouth; *V.b.w.*, ventral body-wall; *Cb.c.*, cardiobranchial (basibranchial) cartilage; *M.cb.*, musculus corcoabranchialis; *L.s.b.*, left suprapericardial body; *V* arch, fifth arch; *P.*, pericardium; *P.g.*, pectoral girdle; *II* to *VI*, second to sixth gill-pouches; *Oes.*, oesophagus.



THE SKULL OF ACANTHIAS VULGARIS¹

GRACE A. WELLS

TWENTY-TWO FIGURES (THREE PLATES)

The studies for the following description of the skull of the spiny dogfish of the Atlantic coast were carried on at the University of Illinois and at the Harpswell Laboratory, South Harpswell, Maine, during the year 1913-1914, under the direction of Dr. J. S. Kingsley.

Although the dogfish *Acanthias vulgaris* (often, but needlessly called *Squalus acanthias*) has been the subject of many studies, there is as yet no adequate account of its skeleton. The skull has been described in a very imperfect manner by Gegenbaur ('72), but his figures are very incorrect. The development of the cranium is given with some detail by Sewertzow ('99). Besides, there are the laboratory directions for the study of the skeleton in the publications of Pratt ('05) and Kingsley ('07). Aside from these I have not found any description of the skull in the literature.

The skull of *Acanthias*, like that of all *Selachii*, is made up of two distinct parts, a cranium (chondrocranium) and a visceral skeleton. The chondrocranium is composed of a median brain case and three lateral pairs of sense capsules, two of which—the otic and the nasal—are fused with the brain case, while the third, the optic or sklera of the eye, is free. The visceral skeleton consists of a series of seven arches loosely connected with the cranium and surrounding the buccal and pharyngeal regions. The adult cranium is without sutures or separate cartilage. It can be treated as exhibiting four surfaces—dorsal, ventral, lateral and basal, the latter being the face which articulates with the vertebral column.

¹ Contributions from the Zoological Laboratory of the University of Illinois, No. 85.

The dorsal surface (fig. 1) is somewhat like the head of a spear in outline, the point of the spear being the anterior end, the so-called rostrum (*r.*). The rostrum is flanked on either side at its base by a nasal capsule (*n.c.*), a rather deep rostro-nasal notch (*r.n.*) intervening between rostrum and capsule. Each nasal capsule is bounded by a shallow vertical groove, limiting the capsule from a strong antorbital process (*p.a.*), the lateral aspect of which will be described later, but which passes, on the dorsal surface of the cranium, into the supraorbital crest (*s.or.c.*). The lateral margin of each supraorbital crest runs obliquely inwards and backwards to the region between and a little posterior to the eyes, and then bends sharply outwards to form the dorso-anterior margin of the strong postorbital process (*p.o.*), which is triangular in outline as seen from above. The sharp dorsal margin of the postorbital process continues back as an acute supraotic crest (*s.ot.c.*) on the dorso-lateral surface of the otic capsule, and then bends obliquely downwards and backwards, terminating at the latero-posterior angle of the skull, in the postotic process (*p.ot.*).

The brain case (*br.*) lies in the median line between the supra-orbital and supraotic crests, and is marked off from the lateral sense capsules by a longitudinal depression—the sulcus longitudinalis (*s.l.*) on either side. Each sulcus begins in front, at the rostro-nasal notch, lateral and ventral to the superior margin of the rostrum, continuing back, as a broad groove, to behind the nasal capsules, where it is narrow and shallow. At about the line of the middle of the postorbital process it is interrupted, but continues again as a groove on the dorso-lateral wall of the otic capsule, finally terminating on the basilar aspect of the skull at the foramina of the ninth nerve (*IX*).

The rostrum is somewhat oval, in a dorsal view, and forms a trough, open above, shallow in front and deeper behind. The opening to the trough is the anterior fontanelle (*a.f.*). Like other sharks the adult *Acanthias* has no posterior fontanelle. The fontanelle is about two and one-half to three times as long as broad. The anterior end of the rostrum has the cartilage walls thickened. The lateral walls of the rostrum are thin, ver-

tical above and then curve downwards and medially to form the floor of the trough. Behind, the fontanelle is bounded by the thin cartilaginous roof of the brain case. The anterior margin of this roof has a deep median notch, and separated from this, on either side, by a slender triangular process, is a lesser notch, the lateral boundary of which passes into the lateral wall of the rostrum. The trough of the rostrum is filled with a loose gelatinous tissue.

On either side and extending outward and posteriorly from the rostral carina is a small cartilage process, the lateral rostral bar (*r.b.*) the 'anhang' of Gegenbaur. Farther back this cartilage passes into a dense gelatinous connective tissue with rather definite boundaries, appearing as dorsal and ventral rami (*dr. vr.*) continuous with the anterior cartilaginous rostral bar, and articulating with the dorsal and ventral walls of the nasal capsule. The extent of cartilage was determined by a study of sections, both longitudinal and transverse, through these bars which were previously stained with borax carmine.

Apparently these rostral bars have not been noticed before in *Acanthias*. Evidently they are the same as the single bar labelled '*r*' by Gegenbaur in his figures of *Galeus*, *Mustelus*, *Prionodon*, *Scyllium* and *Pristiurus* on his plate VIII, and it would seem that they are represented as rudiments in his figures of *Centrophorus* and *Zygaena*, there lettered '*r*.' If this homology be true it follows that what is here called the rostrum, is Gegenbaur's "Medialer Schenkel des Rostrum," (*R*), in his figures. In *Zygaena* the case appears somewhat different, where, owing to the great lateral development of the sides of the skull, carrying the eyes out to the end of the 'hammer,' the nasal capsules are far removed from the median line. In this form the rostrum proper is composed of three bars, these forming the rudimentary sides and floor of the trough. Beside this there is the lateral 'anhang' (*r'*) of Gegenbaur, which would thus correspond to the rostral bar of *Acanthias*.

The postero-lateral part of the cranium is formed by the otic capsules, a postorbital process extending laterally from the antero-lateral angle of each. The foramen magnum (*f.m.*) for the

passage of the spinal cord, is visible from above at the posterior end of the cranium. It lies in the median line, somewhat in advance of the lateral occipital region (to be described later). A little in front of the foramen magnum, a pit—the fossa endolymphaticus (*f.end.*) contains the external openings of the endolymph ducts (*e.d.*). This pit is deepest behind, its boundary curving forward from the posterior side of the fossa until it becomes parallel with the axis of the cranium, gradually fading out just inside the sulcus longitudinalis of either side. The foramina for the endolymph ducts occur, one on either side, close to the wall of the fossa at about the middle of its antero-posterior extent. A little behind these, there is, on either side, a larger opening for the perilymph duct (*per d.*), close to the postero-lateral curvature of the fossa.

Behind the posterior wall of the fossa endolymphaticus, the dorsal surface of the cranium slopes obliquely downward and back to the hinder margin of the cranium, this basal surface being nearly as much vertical as dorsal. From the posterior wall of the endolymph fossa a ridge, the crista occipitalis (*c.o.*), extends in the median line back to the anterior (superior) margin of the foramen magnum.

In the view from above, the posterior margin of the cranium is irregular in outline. Behind and below the foramen magnum is the depression where the centrum of the first vertebra articulates with the cranium and on either side of this is a somewhat triangular occipital condyle (*o.c.*) which articulates with the corresponding side of the centrum. Lateral to each condyle the posterior margin has an arcuate excavation, the outer border of this being formed by the postero-lateral angle of the cranium.

On the roof of either otic capsule, between the fossa endolymphaticus, the postero-lateral angle and the postorbital process are ridges indicating the position of the semicircular canals of the ear, within the capsule. One, over the posterior canal, (*p.c.*) extends from the postero-lateral margin of the fossa endolymphaticus outwards and backwards, fading out near the postero-lateral margin of the cranium. The other, covering the

superior canal (*s.c.*) is practically a continuation of the former—extending forward and outward, nearly parallel with the lateral margin of the fossa endolymphaticus and gradually terminating at about the line of the middle of the postorbital process.

External to the postero-lateral angle and at the level of the floor of the skull, is a notch in the cartilage (see figs. 1, and 7). A cartilage bar continuous at either end with the margin of the notch, converts it into a fenestra postotica (*f.p.o.*). This lies behind and below the glossopharyngeal foramen (to be mentioned below) but the ninth nerve passes outwards and backwards above the fenestra. No nerve, blood vessel or muscle of any size passes through it; but one of the dorsal longitudinal muscles of the trunk has its origin from a fibrous fascia which is attached to the postero-lateral angle of the cartilage bar and the trapezius (*T.*) attaches to the outer angle.

A number of foramina perforate the dorsal surface of the cranium. Some of these are for the passage of nerves, others for blood vessels. Just lateral to the rostrum, is a more or less regular row of small foramina, which extends somewhat diagonally (posteriorly and laterally) on the dorsal surface of the nasal capsule. There are usually four—sometimes only three—of these foramina, the one nearest the rostrum being the largest. As nearly as could be determined after repeated dissections of adult heads and injected garters, these are for minute twigs of the ophthalmicus superficialis ramus of the seventh nerve (*o.s. VII*).

Numerous nerve foramina occur in the sulcus longitudinalis. Posterior and slightly lateral to the above foramina are two small foramina on either side—the inner for the passage of the ophthalmicus profundus (*o.pr.V.*) branch of the fifth nerve and the outer for fibers of superficialis. (Just anterior to the latter a very small foramen sometimes occurs, for the passage of a minute twig of the ophthalmicus superficialis.) These were called openings of the 'ethmoid canals' by Gegenbaur. Posterior to these openings, on either side, is a large foramen for the main trunk of the ophthalmicus superficialis branch of the seventh nerve (*o.s.*) on its way to supply the ampullae of Lorenzini on

the upper surface of the tip of the snout, behind which is a series of minute foramina for the passage of twigs from this nerve (*o.s. VII*) to innervate the lateral line organs in the inter-orbital region. In the postorbital process these foramina are somewhat larger than those more anterior.

Just dorsal and lateral to the postero-lateral angle of the cranium is the foramen of the ninth nerve (*IX*), already referred to. Between this and the occipital condyle is the larger opening for the passage of the main trunk of the tenth or vagus nerve (*X*). Just anterior and slightly lateral to this foramen is a smaller opening for the passage of a branch (probably the first branchial) of the same nerve (*X*).

Posterior to the median notch in the anterior wall of the brain case there is a small heart-shaped opening in the cranial roof. This marks the location of the epiphysis (*e.f.*). In one specimen dissected this was a paired, instead of a single medial opening.

The regions of muscle attachment on the dorsal side of the cranium are restricted to the posterior portion. The trapezius (*T.*) attaches just lateral to the glossopharyngeal (*IX.*) foramen, above the bar of the fenestra postotica; while on the postero-medial angle of the bar is inserted the fibrous fascia of one of the dorsal longitudinal muscles (*D.l.*). Other dorsal longitudinal muscles, corresponding in general to the protractor dorsalis (*Pro.d.*) of Greene and Greene's description of the musculature of the king salmon, attach to the cranial roof between the crista occipitalis and the posterior semicircular canal. Still others have their origin in the triangular depression between the superior and posterior semicircular canals. These muscular attachments are even more clearly seen on the basal surface (fig. 7).

In general outline the ventral surface of the cranium (fig. 2) is much like the dorsal and the same regions may be recognized. In front is the rostrum (*r*) with its strong median carina (*r.c.*) and lateral to this the rostral bar (*r.b.*) with the dorsal and ventral rami of connective tissue referred to in the description of the dorsal surface, only the ventral ramus showing clearly in this view. At the base of the rostrum, at the level of the nasal

capsule, and on either side of the median keel is a fenestra or fontanelle (the 'basal communication' of Gegenbaur) which opens into the brain cavity a little behind the posterior margin of the anterior fontanelle of the dorsal surface.

In *Acanthias* each nasal capsule is almost spherical and is composed of a very thin, easily broken cartilage. In this there are thicker areas, which, at first sight seem to be separate cartilages, but which are really continuous with the rest of the wall. The most marked of these regions is on the ventro-medial part of the capsule, and at its postero-medial edge is a small opening of the so-called 'ethmoid canal.' A second of these denser areas is on the dorso-lateral surface and will be described in connection with the lateral view.

Immediately surrounding the naris (*ext.n.*) is an elliptical cartilage ring, a pointed cartilage bar extending across the short axis of the ellipse from the anterior to the posterior side—continuous with the former, but free posteriorly. This supports the flap of skin which divides the naris into its two functional openings.

Posterior to the nasal capsules a broad antorbital shelf (*a.or.s.*) extends the width of the cranium and slopes postero-ventrally. A continuation of the median rostral carina passes through this as a slight median ridge.

This shelf narrows abruptly so that between the orbits the floor of the cranium has an almost tropibasic appearance, this part, so far as can be judged from Sewertzoff's figures, being developed from the trabecular plates, while the more dorsal part of the orbital wall is derived from the alisphenoid cartilage, the sphenolateral of Gaupp. This interorbital region is not of the same width throughout; it narrows from the preorbital side, then widens, and again constricts (this is the hypophysial region) and then widens again, passing, posteriorly, into the broader basal plate of the interotic region.

The more posterior of these constricted areas is the palato-basal articular surface (*p.b.a.*) of Gegenbaur's description, which is for articulation with the palatal process of the upper jaw. Posterior to this surface the widened area extends farther ven-

trally so as to form a basal angle (*b.a.*, Gegenbaur's 'basalecke'). On the lateral margins of this area there are somewhat crescent shaped elevations which enlarge the articular surface. Antero-lateral to this orbital region is the preorbital process (*p.a.*) and postero-lateral to it, the postorbital process (*p.o.*), the concavity of the orbit lying between these processes.

In the otic region the ventral surface of the cranium is broad and flat (the basal plate), with a slight median ridge extending the length of the region. The ridge over the horizontal semi-circular canal (*h.c.*) is seen just lateral to this flat plate, and posterior to it the fenestra postotica (*f.p.o.*), as described in connection with the dorsal surface, except that the notch in the cranium (region of the ninth nerve) does not show in this view. In the extreme posterior part of the median line there is a crescent shaped depression for articulation with the first vertebra, the triangular occipital condyles (*o.c.*) occurring on either side.

Only a few foramina are seen on the ventral surface. Among these are that for the main trunk of the ophthalmicus superficialis (*o.s.VII*) and the row extending posteriorly for twigs of the same branch, occur on either side of the narrow interorbital region, passing through the base of the supraorbital crest. Medial to these on either side and anterior to the palato-basal articular surface is the optic foramen (*o.II.*). Posterior to the articular surface in the median line is the foramen for the entrance of the united internal carotid arteries (*int.c.*) into the cranium. Lateral and posterior to this median opening there occurs a smaller foramen for the hyoid branch of the facial nerve (*f.VII*). The foramen of the vagus (*X*) nerve is just visible at the posterior margin of the cranium, between the occipital condyles and the fenestra postotica. The foramen for the glosso-pharyngeal nerve can not be seen in a ventral view.

The only notable regions of muscle attachment on the ventral surface of the cranium are those of the levatores labialis superioris which have their origin on either side of the median line, just anterior to the palato-basal articular surface (*L.ls.*). At the extreme posterior end, just in front of the foramen magnum, is

the origin of the first of the medial system of interarcuales (Marion)—the subspinalia of Vetter's description (*Int.arc.I.*).

Except for the triangular rostrum (*r.*), a lateral view of the cranium (figs. 3 and 5) presents a very boxlike appearance, of quite uniform depth and rectangular in general outline, the posterior part being occupied by the otic capsule, the middle by the orbit, and in front of the rostrum.

The median rostral carina (*r.c.*) extends postero-ventrally from the tip of the rostrum to a point medial to the nasal capsule. The rostral bar (*r.b.*) is clearly shown connecting with the carina and its dorsal and ventral connective tissue rami are more distinctly seen in this view than in any other. The somewhat latero-ventral external naris is also visible. A lateral view of the nasal capsule shows the dorso-lateral thickening of the cartilage mentioned in connection with the ventral view. It may be considered a forward extension from the strong ant-orbital process (*p.a.*).

The orbit, in a lateral view, is a large concavity occupying the dorso-ventral extent of the cranium. As mentioned above it is roofed by the supraorbital crest (*s.or.c.*) which passes in front into the ant- and behind to the post-orbital process; these forming, respectively, the anterior and posterior boundaries of the orbit. Along the ventral margin of the orbit are seen, posteriorly, the basal angle (*b.a.*) and just anterior to it, on the narrow interorbital region, the depression of the palato-basal articular surface (*p.b.a.*).

The floor of the cranium slopes upwards from the basal angle to a region just behind the level of the postorbital process and then extends horizontally, so that the depth of the cranium in the otic region is less than in the orbit. A horizontal ridge through about the middle of the otic capsule marks the position of the horizontal semicircular canal of the ear (*h.c.*). Posteriorly this ridge extends toward the fenestra postotica (*f.po.*). Behind the fenestra the triangular occipital condyle is visible (*o.c.*). The middle part of the braincase appears above the level of the supraotic and supraorbital crests. At the base of

the rostrum the triangular process separating the anterior wall of the brain case is visible in profile.

In the lateral view no foramina are seen in the ethmoid region. In the orbit they are numerous. Near the ventral side, at about the middle of the orbit is the large optic foramen (*II*). In the anterior part of the roof is that for the ophthalmicus superficialis (*o.s. VII*) with behind it the seven or eight smaller foramina for its branches to the lateral line organs on the top of the head. Just in front of the postorbital process is the large trigemino-facial foramen for branches of the fifth and seventh nerves (*t.f.*), just in front of which is the oculomotor foramen (*om. III*). About midway between the trigemino-facial and the main ophthalmicus foramina, in the dorsal half of the orbit is the small foramen for the trochlearis (*IV*). Anteroventral and close to the trigemino-facial is the small opening for the abducens. Gegenbaur thought that the abducens left the cranium together with the fifth and seventh nerves. This abducens foramen is, however, mentioned by Allis ('14 b) and I find it as described by him.

Just beyond the abducens foramen is the transbasal canal (*tr.c.*) while just antero-lateral to the main ophthalmicus opening is the foramen for the passage of the ophthalmicus profundus branch of the trigeminal. Below this last is the opening of the so-called ethmoid canal, Reynold's orbito-nasal foramen (*e.c.*). Directly in front of the transbasal canal is a small opening for the passage of a blood vessel which leaves the orbit through a second foramen midway between the optic and the ophthalmicus superficialis foramina. A minute canal perforates the base of the postorbital process, opening on both anterior and posterior surfaces, and associated with other small foramina in the supraotic crest, for the passage of posterior twigs of the superficialis branch of the seventh nerve.

A slight depression just in front of the foramen trigemino-facialis marks the point of attachment of the optic pedicel (*o.p.*). This is a cartilaginous stalk with a rotule at the distal end for the support of the eyeball; it is not continuous with the sklera of the eye, but is held within the connective tissue which covers the ball.

In the anterior part of the floor of the otic region are two foramina, separated by a rather prominent projection, through which pass the hyomandibular branches of the facial nerve (*hym.*) In the extreme posterior part of the otic region, just above the fenestra postotica is the notch in the cranium in which is the glossopharyngeal foramen (*IX*).

Besides the points of origin of the eye muscles, there are two important regions of muscular attachment on the lateral surface of the cranium. One of these, just behind the postorbital process and below the supraotic crest, is the origin of the levator maxillae superioris (*l.m.s.*). Posterior to this and just below the postotic process is the origin of the second constrictor superior dorsalis (*csd.2*).

The basal surface of the cranium presents little not already described (fig. 7). Ventrally, in the middle line is the articular surface for articulation with the centrum of the first vertebra, and just lateral to it, on either side, is an occipital condyle (*o.c.*). Above these is the foramen magnum (*f.m.*), from which the crista occipitalis extends to the posterior margin of the fossa endolymphaticus (*o.c.*). Laterally is the elevation over the posterior semicircular canal of the ear (*p.c.*). Near the posterolateral angle the fenestra postotica (*f.po.*), and the foramina for the ninth (*IX*) and tenth nerves (*X*) are visible.

Figure 4 shows a median section and the inner surface of half of the cranium. The roof is rather uniform in thickness, except for a marked thickening just behind the endolymph fossa and a lesser one behind the anterior fontanelle. The floor, however, shows marked variations in this respect, being thicker below the regions of pro- and mesencephalon than below the posterior regions of the brain. The section of the rostral carina makes that part appear thicker. The floor rises in the posterior part of the cranium, and in this part the notochord (*nch.*) runs, from the posterior end of the skull forward to the dorsum sellae, turning dorsally to enter this projection. Just anterior to the dorsum (*d.s.*) is a rather deep pit, the fossa hypophyseos (*f.h.*) or sella turcica, in which the infundibulum, hypophysis and associated structures lie.

This figure shows well the trough of the rostrum and its median keel. Ventral to the notch at the anterior end of the brain case is an opening through to the ventral side of the cranium, just medial to the nasal capsule. This is the fenestra, the basal communication of Gegenbaur, mentioned in the account of the ventral surface. Dorsal and posterior to this is the opening leading into the nasal capsule, through which the olfactory tract passes (*o.n.c.*).

Slight ridges in the internal cranial wall mark off the brain cavity approximately into thirds. The most anterior of these regions is that in which the prosencephalon lies and the only foramen here is a small one in the postero-dorsal part of the region for the passage of a blood vessel (*b.*), the relations of which were not determined. The central region, the location of mes- and metencephalon, corresponds in general with the external orbit. At its lower anterior angle is the large optic foramen (*o.II*). Just anterior to this is a slight thickening, the presphenoid process (*Praesphenoid Vorsprung*) of Gegenbaur's description. At the upper anterior angle of this region is the small foramen for the trochlearis nerve (*IV*). In the posterior part of this region, near the floor of the cranium, is the canal for the entrance of a blood vessel into the orbit (*b.*). Just dorsal to this is the foramen of the oculomotor nerve (*o.m.*). At the base of the dorsum sellae, in the floor of the cranium, is the foramen for the transbasal canal (*tr.c.*) and ventral to it, and slightly anterior, is the canal for the internal carotid artery (*int.c.*).

In the anterior part of the posterior region there are three large foramina in a common depression (the acustico-trigeminofacialis recess of Allis). The most anterior of these is the foramen trigeminofacialis (*t.f.*), the most posterior is that of the glossopharyngeal (*IX*), while the middle foramen is for the auditory nerve (*VIII*). Gegenbaur called the most posterior of these the auditory foramen, and the middle one, the facial, but probing from the basal opening of the ninth shows that it leaves the brain case through the most posterior of the three

openings, while sections of the labyrinth region show that the central opening is that of the eighth nerve. A dissection of the cranium in this region was also made, following along the nerve canals.

Ventral to these foramina and below their common recess is a small opening for the sixth (abducens) nerve (VI). This opening lies between the trigemino-facialis foramen and the foramen of the eighth nerve. Behind the foramen for the main trunk of the glossopharyngeal is a small opening for a second branch of the same nerve (IX); and posterior to this is the large foramen for the main trunk of the vagus nerve (X), with a small opening for a minute branch just behind it.

Above the cut edge of the cranial roof in this region (that of the fossa endolymphaticus) is, posteriorly, the perilymph duct (*per.d.*) and anterior to it—the opening of the endolymph duct (*e.d.*).

The labyrinth region was studied by means of transverse sections through the otic capsule of the left side of an adult cranium (figures 11 to 22 inclusive). These were free hand sections about 2 mm. in thickness. In each case the anterior side of the cut was drawn, twice the natural size. Only the skeletal labyrinth is represented, no attempt being made to show the membranous parts. The primitive condition of the membranous labyrinth of *Acanthias* is evident, however, from the corresponding simplicity of the skeletal labyrinth. This is well shown in sections 16, 17 and 18. The vesicle is practically a single chamber, with only suggestions of a constriction into saccular and utricular regions. The connections of superior, horizontal and posterior ampullae with the otic chamber are seen in sections 13, 14 and 19 respectively. In sections 15 to 18 inclusive, the dorsal part of the auditory vesicle is occupied by the perilymph cavity, the divisions being membranous and so not shown in the drawings.

The visceral skeleton consists of a series of seven cartilaginous arches so arranged as to form the skeletal elements of the mouth and the support of the gill clefts (figs. 5 and 6). The

general structure of these arches is best shown in a ventral view (fig. 6) the arches themselves being laid open as much as possible and still retain their connections.

The most anterior arch, the mandibular (*md.*) is the largest and the most modified (see also fig. 9). It is divided into a dorsal and a ventral portion, these articulating with each other posteriorly, and forming the upper and lower jaws. The upper is the pterygoquadrate (palatoquadrate), the lower is Meckel's cartilage. Each is composed of right and left halves which are articulated in the middle line.

The pterygoquadrate is composed of two curved and vertically flattened bars, thinnest anteriorly where they meet in the middle line. At about the anterior third each bears a strong palatine process on its upper border (*p.p.*) which plays against the palato-basal articular surface of the cranium. From this point the bar narrows posteriorly and then expands into the quadrate portion bearing above an expansion, concave externally for the attachment of the adductor mandibularis muscle. From this an articular process extends downwards and backwards and bears on its distal extremity a convex surface with which Meckel's cartilage articulates. On its anterior surface the pterygoquadrate is convex from above downwards; on the posterior or oral side the anterior two-thirds presents a marked alveolar groove, in which the functional and reserve teeth are situated. The ventral margin of the groove is sharp.

The Meckelian cartilage is thin, curved in the horizontal plane, and gradually increasing in height from the symphysis to near the posterior angle. On the oral side there is an alveolar groove, similar to and of about the same extent as that in the upper jaw. The inferior border of this groove extends obliquely upwards and backwards and is continued into a strong coronoid process which connects distally with the lower anterior angle of the hyomandibular cartilage. Just behind this process the lower part of the Meckelian curves strongly outwards into an angular process, on the upper surface of which is a concave facet for articulation with the pterygoquadrate. Below this the process ends in a right angle. On the outer side of the jaw

is an oblique ridge extending from the coronoid process forwards and downwards and marking the anterior limits of the insertion of the adductor mandibularis muscle.

Connected with the jaws are the labial cartilages, these being imbedded in the muscular and other tissue external to the upper jaw (fig. 9. l^1 , l^2). The larger pair (l^2) extends laterally from near the pterygoquadrate symphysis to a point nearly below the expansion for the adductor muscle where it connects with a ventral element. The second labials (l^1) are imbedded in a similar way on the anterior face of the Meckelian cartilage, though they are far less extensive.

Between the mandibular and the second (hyoid) arch is the spiracle, the anterior wall of which is supported by two small flat cartilage bars (fig. 8) which are connected by a broad ligament, which in turn, is attached to the cranium under the levator maxillae superior muscle (*l.m.s.* figs. 3 and 5) on the dorsal portion of the postorbital process.

The second or hyoid arch consists of a pair of cartilage rods attached at their dorsal ends to the cranium and united ventrally by a broad unpaired plate of cartilage, the basihyal (*b.hy.l.*). Each half of the hyoid arch is divided into two bars, comparable, in a way, to the pterygoquadrate and Meckel's cartilage of the mandibular arch. These halves are, a dorsal hyomandibular (*hy.m.*) which articulates with the cranium, and a ventral ceratohyal (*ch.*). The hyomandibular is relatively short and stout, and acts as the suspensor of the jaws. Its upper end has a slight knob-like thickening and it curves somewhat medially, to articulate with the postero-lateral surface of the cranium in the region of the horizontal semicircular canal. The lower end, antero-dorsal to its connection with the ceratohyal, has a flat thickened area for the attachment of a ligament connecting the hyomandibular cartilage with the lower jaw. The hyomandibular bears gill rays (*g.r.*) on its postero-ventral margin and has depressed surfaces for muscle attachment. Its distal end connects with the ceratohyal, a long slender bar (also bearing gill rays on its postero-ventral margin), which articulates with the median basihyal (*b.hy.*). The latter is a

broad plate bearing two processes, one extending laterally on the postero-dorsal edge, and the other projecting on the antero-ventral margin; these are for articulation with the ceratohyal.

The five brancial arches are all very similar in structure, each half arch consisting of four pieces of cartilage, so connected with the other half as to form a hoop-like support (incomplete dorsally) for the pharyngeal region. The most dorsal element of each arch is the pharyngobranchial cartilage (*ph.br.*) a flattened, pointed plate with its free inner end extending inwards and backwards beneath the vertebral column. The fourth and fifth pharyngobranchials are united at their distal ends, and there are longitudinal grooves on the dorsal basal ends of the second, third and fourth of these elements, which are for the passage of blood vessels (*b.*) to the gill folds. (In one specimen the grooves terminated in a foramen in the fourth and fifth pharyngobranchial of one side).

At its ventral end each pharyngobranchial articulates with a short epibranchial (*e.br.*) bearing gill rays on its posterior border. These elements are flattened and each has its dorsal end expanded where it articulates with the pharyngobranchial.

Ventrally the epibranchials are succeeded by the longer ceratobranchials (*c.br.*), each bearing similar gill rays. At their medial or ventral ends the five pairs of ceratobranchials articulate with each other, and the four anterior are connected medially with three pairs of short hypobranchials (*hy.br.*) the relations of these latter being as follows:

The anterior pair of hypobranchials are connected with the first pair of ceratobranchials. They bend inwards and backwards below the pharynx and meet in the middle line. Posteriorly they articulate with a median plate of cartilage, the first basibranchial (*b.br.¹*). The second pair of hypobranchials are related in a similar way to the second ceratobranchials and meet each other in the median line, while the first basibranchial is included between their anterior margins. Posteriorly they abut against the second basibranchial to be described in a moment. The third pair of hypobranchials articulate laterally with the third and fourth ceratobranchials, while medially they

meet the second hypobranchials on their lateral (posterior) sides, and also they articulate with the second basibranchial.

The fifth ceratobranchial is wider than its fellows and bears plate-like expansion, directed laterally, on its posterior border, while anteriorly there is a strong process or tubercle. This arch lacks a hypobranchial, the ceratobranchial articulating directly with the second basibranchial.

The second basibranchial is a flattened triangular plate of cartilage, the apex of the triangle directed posteriorly. At about two-thirds the distance towards the posterior angle, it is slightly constricted, and from this line a shallow groove runs forward in the median line of the dorsal surface.

Thin plates of cartilage, the extra-branchials occur on the outer sides of the gill arches. Of these there are two series, a dorsal and a ventral, the former parallel with the epibranchial elements, the ventral bearing the same relations to the ceratobranchials. These occur in each arch, but the anterior extra-branchials are so small that they may easily be overlooked. These cartilages serve as points of attachment for the posterior four dorsal constrictor muscles.

The regions of muscular attachment on the visceral skeleton are somewhat numerous. The adductor mandibulae (fig. 5, *am.*) has its origin from the quadrate portion of the pterygoquadrate bar; it extends ventrally and posteriorly and, then, curving forward, is inserted on the depressed posterior external surface of Meckel's cartilage. The first constrictor superior dorsalis (*csd. 1*) is inserted on the more medial region of the hyomandibular, while the anterior fibers of the second (*csd. 2*) are inserted on it, near its articulation with the ceratohyal. (These were described as attached to the upper jaw by Marion, but this was not the case in the specimen I examined.) Other more lateral fibers of this muscle are inserted on the posterior edge of the ceratohyal. The first constrictor superior ventralis (*csv. 1*.) is inserted along the postero-ventral margin of Meckel's cartilage, while the second, (*csv. 2*.) has its insertion on the ventral edge of the ceratohyal. The levator maxillae superioris (*lms.*) is inserted on the pterygoquadrate bar of either side,

just postero-ventral to the palatal process (*p.p.*) and on either side of the symphysis. Its origin is described in connection with the account of the lateral surface of the cranium. The series of five interbranchials have their insertions on the anterior margin of the cerato- and epibranchials (*int.br.1-5*). The insertion of the first of the medial system of interarculae (*m.int.arc.1*) has already been mentioned in connection with the ventral surface. The remaining four (*m.int.arc.2-5*) are inserted on the postero-medial surface of the pharyngobranchials. Each of the series of the lateral system of interarculae (*l.int.arc.1-5*) is inserted, partly on the proximal end of its pharyngobranchial, and the remaining fibers on a contiguous area on the epibranchial.

The coracomandibularis (*am.*) has its origin in the ventro-posterior surface of the lower jaw, in a restricted area on either side of the symphysis. The coracohyoiedus (*Chy.*) is inserted along the postero-ventral margin of the basihyal, while the first of the series of coraco-branchials (*c.br.*) has its insertion on the medial edge of the ceratohyal. The remainder of the series is inserted on the proximal ends of the last four ceratobranchials.

A small muscle, the adductor arcus branchialis (fig. 10, *Aab.*) is attached on the internal surface of each gill arch at the lateral hinge, one end being inserted on the distal edge of the ceratobranchial, while the other is attached to the adjacent end of the epibranchial. This small muscle belongs in the same series as does the large, adductor mandibularis, mentioned above.

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ABBREVIATIONS

<i>aab.</i> , adductor arcus branchialis	<i>hy.m.</i> , hyomandibular
<i>a.f.</i> , anterior fontanelle	<i>int.arc. 1-5</i> , interarcuales; <i>m.</i> , medial system; <i>l.</i> , lateral system
<i>a.m.</i> , adductor mandibularis (in fig. 6, coracobranchialis)	<i>int.br. 1-5</i> , interbranchiales
<i>am.h.c.</i> , ampullar region of horizontal semicircular canal	<i>int.c.</i> , internal carotid
<i>am. p. c.</i> , ampullar region of posterior semicircular canal	<i>l.1-2.</i> , labial cartilages
<i>a.or.s.</i> , antorbital shelf	<i>lg.</i> , spiracular ligament
<i>a.p.</i> , antorbital process	<i>lls.</i> , levator labialis superioris
<i>au.v.</i> , auditory vesicle	<i>lms.</i> , levator maxillae superioris
<i>b.</i> , foramen for blood vessel	<i>M.</i> , Meckel's cartilage
<i>b.a.</i> , basal angle	<i>md.</i> , mandibular arch
<i>b.br.</i> , basibranchial	<i>n.c.</i> , nasal capsule
<i>b.hy.</i> , basihyal	<i>nch.</i> , notochord
<i>br.</i> , brain case	<i>o.II.</i> , optic foramen
<i>c.</i> , canal for carotid artery	<i>o.c.</i> , occipital condyle
<i>cbr.</i> , coraco-branchialis	<i>om.III.</i> , oculomotor foramen
<i>cer.br.</i> , ceratobranchial	<i>o.n.c.</i> , opening into nasal capsule
<i>cer. hy.</i> , ceratohyal	<i>orb.</i> , orbit
<i>c.hy.</i> , coraco-hyoideus	<i>o.p.</i> , optic pedicel
<i>cm.</i> , coraco-mandibularis	<i>o.pr.</i> , foramen for ophthalmicus profundus
<i>c.o.</i> , crista occipitalis	<i>o.s.</i> , foramen for ophthalmicus superficialis
<i>cr.c.</i> , cranial cavity	<i>p.b.a.</i> , palato-basal articular surface
<i>cs.</i> , spiracular cartilage	<i>p.c.</i> , posterior semicircular canal
<i>csd. 1-6</i> , constrictores superiores dorsales	<i>per.d.</i> , perilymph duct
<i>csv. 1-6</i> , constrictores superiores ventrales	<i>ph.br.</i> , pharyngobranchial
<i>dl.</i> , dorsal longitudinal muscles	<i>p.o.</i> , postorbital process
<i>dr.</i> , dorsal ramus of rostral ligament	<i>p.ot.</i> , postotic process
<i>d.s.</i> , dorsum sellae	<i>p.p.</i> , palatine process
<i>e.br.</i> , epibranchial	<i>p.q.</i> , pterygoquadrate bar
<i>c.c.</i> , ethmoid canal	<i>pro.D.</i> , protractor dorsalis
<i>e.d.</i> , endolymph duct	<i>q.p.</i> , quadrate process of pterygoquadrate
<i>e.f.</i> , epiphysial foramen	<i>r.</i> , rostrum
<i>ext. n.</i> , external naris	<i>r.b.</i> , rostral bar
<i>f.VII</i> , foramen of facialis branch of seventh nerve	<i>r.c.</i> , rostral carina
<i>f.end.</i> , fossa endolymphaticus	<i>r.n.</i> , rostro-nasal notch
<i>f.h.</i> , fossa hypophyseos	<i>s.c.</i> , superior semicircular canal
<i>f.m.</i> , foramen magnum	<i>s.l.</i> , sulcus longitudinalis
<i>f.po.</i> , fenestra postotica	<i>s.or.c.</i> , supraorbital crest
<i>g.r.</i> , gill rays	<i>s.ot.c.</i> , supraotic crest
<i>h.c.</i> , horizontal semicircular canal	<i>t.</i> , trapezius
<i>hy.</i> , hyoid arch	<i>t.f.</i> , trigemino-facial foramen
<i>hy.br.</i> , hypobranchial	<i>tr.c.</i> , transbasal canal
	<i>v.r.</i> , ventral ramus of rostral ligament
	<i>I-IX</i> , foramina of cranial nerves.

In all the figures parallel oblique red lines indicate the points of attachment of muscles.

PLATES

PLATE 1

EXPLANATION OF FIGURES

- 1 Dorsal view of cranium
- 2 Ventral view of cranium
- 3 Left side of cranium
- 4 Median section and internal surface of cranium

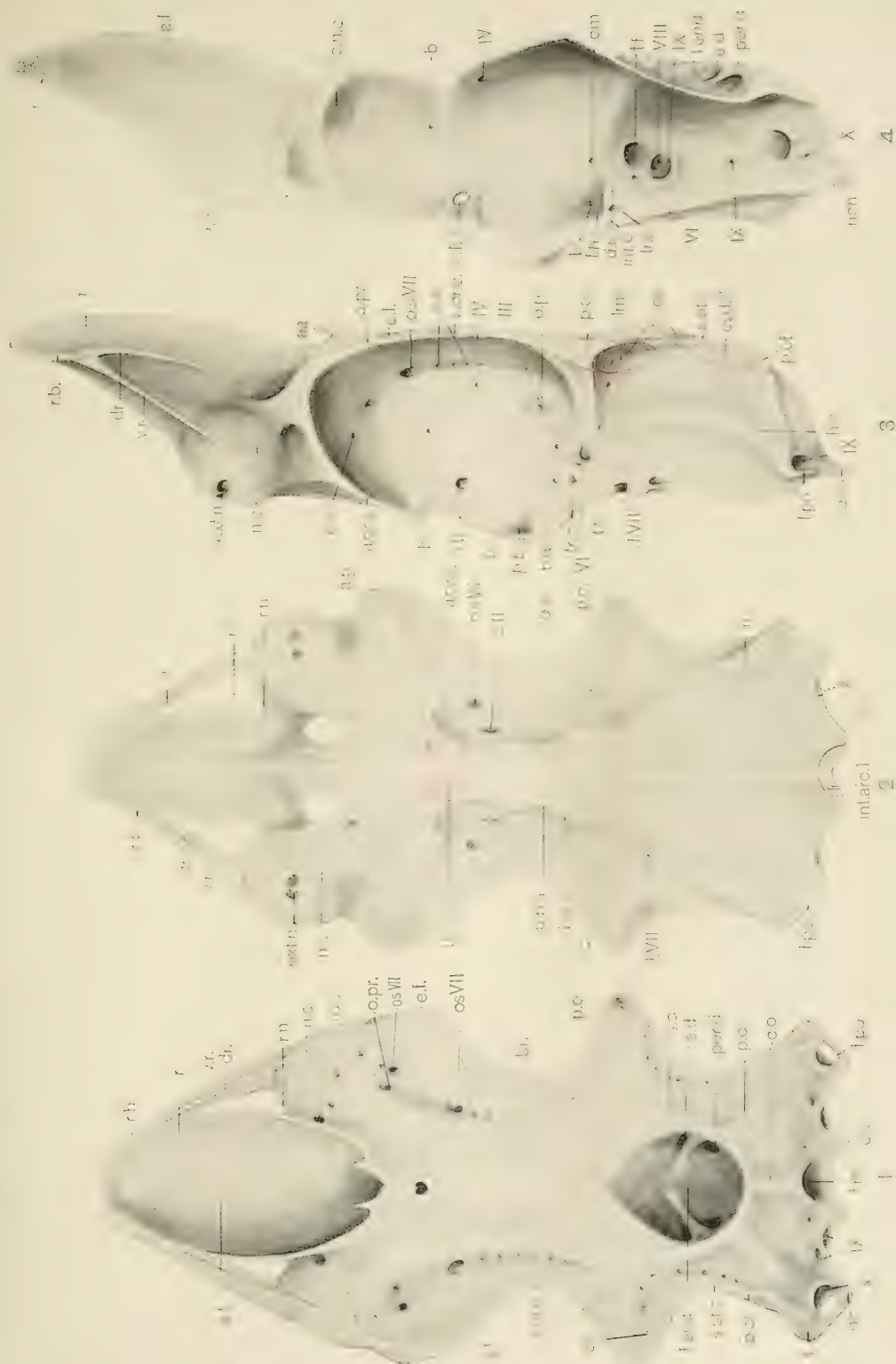


PLATE 2

EXPLANATION OF FIGURES

- 5 Lateral view of whole skull with points of muscular attachment
- 6 Visceral arches, ventral view. (The arches spread out so as to show all parts.)
- 7 Basal view of skull
- 8 Cartilages and ligament in anterior wall of spiracle
- 9 Mandibular arch and labial cartilages from in front
- 10 Cerato-, epi-, and pharyngobranchials

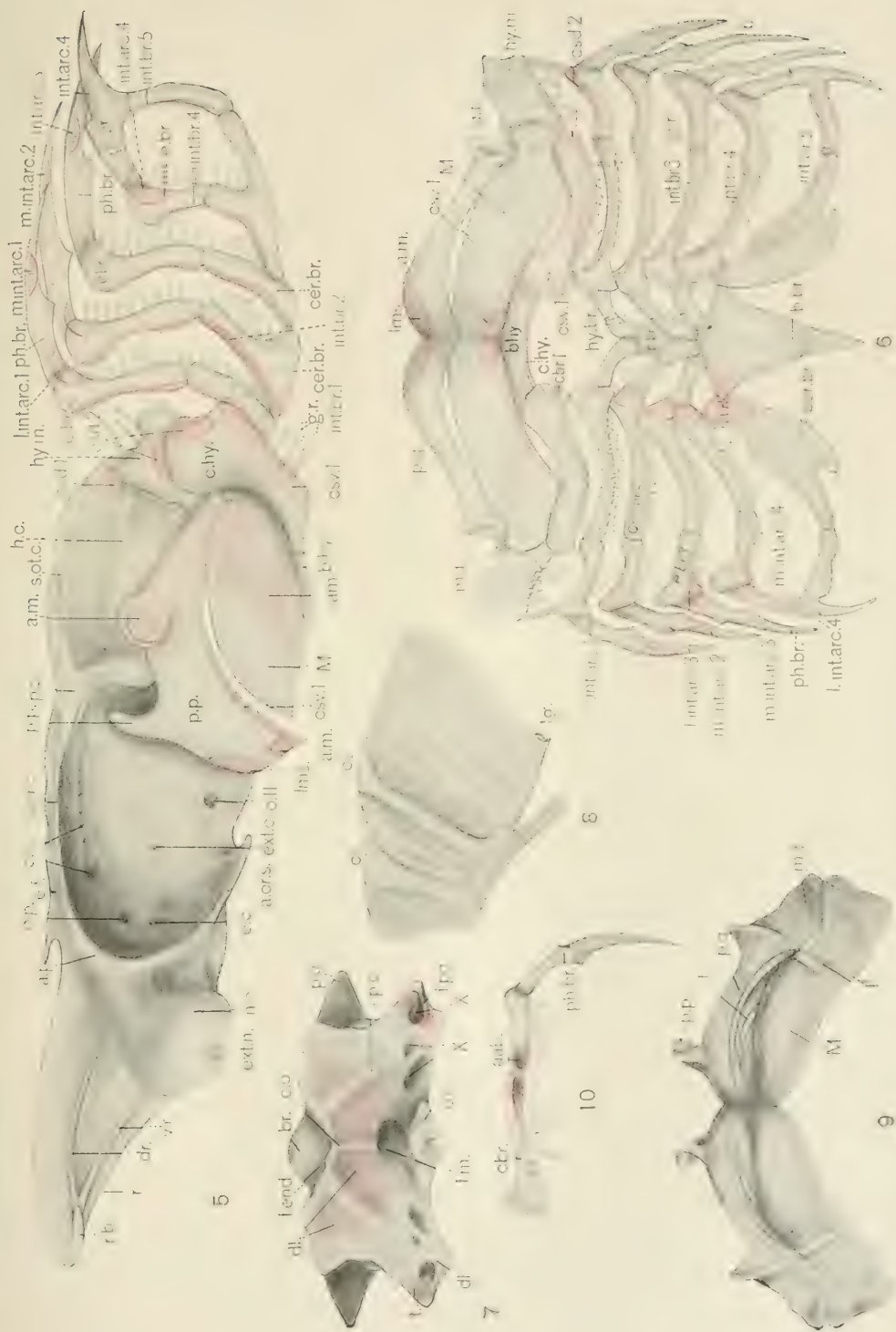


PLATE 3

EXPLANATION OF FIGURES

- 11 to 22 Successive sections, about 2 mm. apart, through the otic capsule
- 11 Passing through the anterior end and through the trigeminofacial foramen
- 12 Through center of postorbital process
- 14 Just anterior to endolymph duct
- 16 Through endolymph duct
- 17 Through perilymph duct
- 19 Through anterior part of horizontal canal
- 21 Through posterior end of horizontal canal and canals for nerves IX and X
- 22 Through posterior loop of the posterior canal.

THE SEGREGATION AND RECOMBINATION OF HOMOLOGOUS CHROMOSOMES AS FOUND IN TWO GENERA OF ACRIDIDAE (ORTHOPTERA)

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FIVE TEXT FIGURES AND FOURTEEN PLATES

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¹ Contribution from the Zoological Laboratory of the University of Pennsylvania.

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I. INTRODUCTION

1. *Statement of the problem*

The present trend towards a closer correlation of the subjects of cytology and genetics is one that promises much mutual benefit, as is well demonstrated by the work of T. H. Morgan and his co-laborers at Columbia University. While the present work deals entirely with cytological data, the material promises much for a genetical study which, it is hoped, may be undertaken later.

Early in the course of his studies on Acrididian spermatogenesis Dr. McClung outlined a general plan of study which, it is hoped, will lead to a correlation between chromosomes and body characters. This plan involves (1) a study of the chromosomes of selected species of closely related genera; (2) a study of numerous species within a genus; and (3) a study of the details of organization of the chromosomes of certain species.

In pursuance of this plan McClung has worked on the genus *Hesperotettix*; Nowlin, taking up the genus *Melanoplus* belonging to the same group, has published ('08, '14) her results on five species of this genus; Robertson ('16) has carried on work on the Tettiginae; Pinney ('08) and Wenrich ('16) have studied the genus *Phrynotettix*; and the writer has begun work on the genus *Trimerotropis*—subfamily Oedipodinae.

It is my purpose to confine this paper as closely as possible to its own special topic. Two hypotheses, put forward by Van Beneden ('83) and elaborated by Boveri ('88, '02) are, however, essential to an interpretation of the facts presented. The first, that of the individuality of the chromosomes, also supported by Rabl ('85), has been actually demonstrated for the individual.

at least, in one of the short-horned grasshoppers, *Phrynotettix magnus*, by Wenrich ('16) who, through years of close study, has been able to follow one well marked individual chromosome pair from the spermatogonia through the most diffuse stages to the spermatids. The other one-time hypothesis of Van Beneden ('83) that one-half of the chromosomes are of paternal and one-half of maternal origin was clearly shown to be a fact by the late Carl Mulsow ('12) in his study of the gametogenesis and fertilization of a parasitic trematode, *Ancyracanthus*, in which the chromosomes may be counted, even in the living spermatozoon as it enters the egg and fuses with the female pronucleus.

Sutton ('02) was the first to furnish actual support for the latter hypothesis and to suggest a thorough-going correlation of the chromosomes and somatic characters; he recognized in *Brachystola magna* a double series of chromosomes in the spermatogonia which he considered to be of biparental origin. He indicated the probable relationship between Mendelian phenomena and the possible distribution of the chromosomes in the gametes and their recombination in the zygote. But, so long as the homologous chromosomes of this double series were indistinguishable, it was impossible to follow, cytologically, the chromosomes derived from a given parent and to determine their manner of segregation in relation to any other chromosomes.

As is well known, in the Orthoptera and many other animals as well, the first maturation division determines which of the derivative cells will, in fertilization, produce a male and which a female, since the accessory chromosome—the sex determinant—passes undivided into one of the daughter cells at this time. While probably it is purely a matter of chance which of the daughter cells the accessory enters—that is, it parallels any Mendelian character in the matter of segregation—nevertheless, it marks unalterably, after it has passed to one pole, the male from the female producing spermatozoon. If one of the homologues of any pair is recognizably different from its mate and these homologues should segregate from each other at the first maturation division their manner of segregation in relation to sex would be apparent. The only instances of this sort to be

reported, so far, are those in which the homologues differ markedly in size. The first was the work of the writer which appeared in 1913, giving data showing the alternative distribution of the unequal dyads of one tetrad in relation to sex in certain Oedipodinae. Three hundred first spermatocytes were counted; in 51.3 per cent the larger dyad was going into the same second spermatocyte as the accessory, and in 48.7 per cent the smaller dyad was accompanying the accessory. Shortly afterward a paper by Vořnov ('14) appeared, giving like results and an essentially similar conclusion from a study of *Gryllotalpa vulgaris*.

Wenrich, in 1914, reported similar results from a count of four hundred and seventy-two first spermatocytes of *Phrynotettix magnus* where a tetrad with unequal dyads is also present. And lastly, Robertson ('15), in an addendum to a paper in which he reports an unequal pair of chromosomes in *Tettigidea parvipennis* and in *Acridium granulatum*, states that these unequal pairs agree with the one in my material in regard to the distribution of their dyads in relation to the accessory. These I believe are the only instances so far reported where the chromosomes derived from different parents could be followed in the germ cells and their distribution determined.

It is to this problem of tracing the segregation of certain well marked homologues in given individuals of the species studied and then of determining the combinations actually present in a considerable number of individuals of these species that I wish to devote this paper. My study has been confined to the metaphases, but a knowledge of the behavior of the dissimilar homologues during synapsis is essential to my conclusions. As this problem was aside from my general plan, and directly in line with Dr. Wenrich's work, he kindly undertook its solution. In a paper now in press he shows that the process of synapsis and tetrad formation is the same for the heteromorphic tetrads as for the homomorphic ones, whether the latter have terminal or nonterminal fiber attachment.

2. *Nomenclature*

The unusual conditions of the chromosomes in this group have made advisable the introduction of four new terms.

1. Homomorphic—used to designate those tetrads made up of morphologically similar homologues.

2. Heteromorphic—used to designate those tetrads made up of morphologically different homologues.

3. Telomitic—a term used to indicate terminal fiber attachment.

4. Atelomitic—a term used to indicate nonterminal fiber attachment.

The two latter are extensions of the ideas involved in the terms 'Hippiscus type' and 'Stenobothrus type' as used by McClung ('14). This has seemed desirable since the work of Robertson ('16) and some recent work of McClung indicates that the chromosomes of *Stenobothrus* may be fundamentally different from those of the typical *Acrididian* complex. I shall also apply the terms telomitic and atelomitic to spermatogonial and somatic chromosomes as well as to those of the first spermatocyte. The term Hippiscus type will be used interchangeably with telomitic for those first spermatocyte chromosomes which are comparable in structure to the Hippiscus rings. However, in this material the tetrads of the Hippiscus type are usually transformed into rods in the metaphase as described by McClung ('14). In the same way *Stenobothrus* type and atelomitic may be used interchangeably.

3. *Special fitness of material*

The genus *Trimerotropis* is confined to the American continent and contains over thirty species, the extreme members of which merge with those of four other genera. The present paper deals with the fallax group of the genus and its relation to the genus *Circotettix*, with especial reference to the heteromorphic tetrads found in each. In regard to the systematic relationships, it is believed that a definite cytological criterion—

difference in number of chromosomes—has been found for separating the two genera.

On this evidence the species *suffusus* belongs to the genus *Trimerotropis* instead of to *Circotettix*, while the heteromorphic tetrads found in both genera may point to a common origin. Furthermore, the evidence, as will be shown, indicates that the *fallax* group constitutes a single species.

The group which forms the basis of this work is known to taxonomists as one of the most difficult to classify. Certain species of the group have been removed to the genus *Circotettix* by some taxonomists, while others have split up the remainder into four species. If one assume that the chromosomes are direct descendants from ancestors morphologically like themselves, are the bearers of the determiners of the hereditary characters, and that the homologues actually express, in their architecture, differences in the determiners, the results of a study of the maturation divisions in the male leave little surprise that taxonomists are bewildered.

Dr. McClung has shown ('14) that the point of spindle fiber attachment is normally constant, but at some period in the history of this species there must have been a reorganization to the extent of a shifting of the attachment in certain chromosomes. The most striking result has been to produce J-shaped tetrads in the first spermatocyte. Such shapes are due to one homologue being rod-shaped; that is, it has terminal fiber attachment, while its mate has nonterminal fiber attachment. Of the twelve first spermatocyte chromosomes, ten tetrads and the accessory are affected by this reorganization. If the female is similarly involved and if there is free fertilization, bringing about chance recombination, one might expect to find in a given individual all ten of the tetrads represented by two rod-shaped homologues, giving rod-shaped tetrads, or one might find all of the homologues with nonterminal fiber attachment, resulting in tetrads of the *Stenobothrus* type (McClung '14). Between these two extremes there might be present every possible combination in J's, rod's and *Stenobothrus*-like rings.

These expectations have been realized in part; and, furthermore, in two of these ten tetrads there is a third type with both homologues having nonterminal fiber attachment, but with a secondary shifting so that one of the free arms is longer than the other. One finds, besides such heteromorphic tetrads (plate 14, fig. 22c), homomorphic tetrads of the two expected types, one with both arms long (plate 14, fig. 23a) and the other with both arms short (plate 14, fig. 10a).

Another peculiarity, that of a single constriction occurring near the proximal ends (that is, the ends directed towards the poles), may mark certain tetrads. An example may be seen on plate 14, figure 21a. One dyad of such a tetrad (Chromosome number 3, plate 4) may be of three types; V-shaped (plate 5, fig. 31), plain rod (plate 5, fig. 32) or constricted rod (plate 5, figs. 31 and 32); these occur in various combinations, furnishing a visible mechanism for possible triple allelomorphs.

4. *Methods and acknowledgments*

The material for the somatic mitoses of the female was fixed in a modification of Bouin's solution which has been developed in this laboratory and which promises to be very useful. The following is the formula:

Picric acid, saturate aqueous solution.....	75 cc.
Formalin (full strength).....	15 cc.
Glacial acetic acid.....	10 cc.
Urea, crystals.....	$\frac{1}{2}$ gm.

After immersion in this fluid, material may be removed any time between twenty-four hours and three months. It should then be washed in water some fifteen minutes and run up gradually to 75 per cent alcohol where it may be kept indefinitely. The fact that specimens may be left for a long time in this fixative makes it especially desirable for field work and for those not equipped to make use of more elaborate methods. Acrididian metaphase chromosomes are as well preserved as in Flemming.

The testes were fixed in strong Flemming solution. Sections were cut from 7 to 12 microns in thickness; 10 proved best suited to this work. The stains used were Flemming's tricolor and Heidenhain's iron-hematoxylin.

I wish to thank Dr. McClung for many helpful suggestions and much kindly interest as well as for material from his own collection. It is also through his efforts that I am indebted to the University of Pennsylvania for a collecting trip during the summer of 1915 to obtain additional material. I am, besides, indebted to the Marine Biological Station at Woods Hole for laboratory facilities during the summer of 1914. I also wish to express my thanks to Mr. J. A. G. Rehn and Mr. Morgan Hebard of the Academy of Natural Science for the identification of the specimens and helpful suggestions concerning the taxonomy of the group, and finally to Dr. D. H. Wenrich for much kindly cooperation.

II. OBSERVATIONS

Since the evidence for segregation and recombination must be derived largely from the maturation divisions, I shall begin with the first spermatocytes, then go back to the spermatogonia and to the somatic complexes of the female for corroborative evidence, and finally, take up the second spermatocytes, which are morphologically related directly to the spermatogonial and somatic complexes. The individuals are numbered consecutively. Where more than one complex is shown from an individual the succeeding ones are indicated by letters; e.g., number 1 always refers to a given animal; the letters after the 1 (*a to x*) designate drawings from this specimen.

1. First spermatocytes

a. Segregation of homologues of J-shaped tetrads in individual no. 1, Trimerotropis (?) suffusa (?). This is one of two individuals belonging to what I designate as form 'B.' They were collected on Orcas Island in Puget Sound by a party from Kansas University in the summer of 1909. Both individuals are alike.

Of the twelve first spermatocyte chromosomes (plate 1), four (nos. 9 to 12) are atelomitic—*Stenobothrus* type (McClung, '14)—four (nos. 2, 3, 5, and 6) are telomitic—*Hippiscus* type, the accessory (no. 4) is V-shaped, atelomitic, while the remaining three tetrads (nos. 1, 7 and 8) have one dyad of the *Stenobothrus* type and the other of the *Hippiscus* type.

Several important questions at once present themselves. 1) Since these heteromorphic but homologous dyads segregate in the first maturation division, what is their distribution in relation to the accessory (that is, to sex)? 2) As it is obvious that at least sixteen sorts of spermatozoa are formed in these two individuals, would the well known constancy of the complex—as shown by numerous workers on Orthoptera—hold for this species or would a large number of individuals give all possible combinations? Or would certain combinations result and others fail? 3) Would the somatic complex of the female be constant? (Using this as an index of the oogonial complex). 4) If the complex is constant for the species, what is the mechanism by which it is regulated? Is there selective fertilization of a most complex sort, or is there free fertilization with regulation occurring at the time of the maturation of the egg? (Since copulation occurs some twenty-four hours before the polar bodies are formed the latter seemed quite possible.)

The first of these questions was a matter to be determined by a study of a given individual and was at once worked out from the material in hand. The others required a considerable number of individuals and it was largely for the purpose of obtaining these that a collecting trip through the southern and western states was undertaken during the summer of 1915.

For a determination of the method of segregation of the heteromorphic homologues in relation to the accessory, one hundred camera lucida drawings of entire complexes were made at random. As stated above, the two individuals in the collection were alike; fifty-seven of the drawings were from one individual and forty-three from the other. Plate 1 is based on only one individual, however, but is representative of both.

These drawings are from sections. The chromosomes from one cell are always in two and sometimes three sections. The sections were all present, in order and in straight rows, so that the problem of identifying in successive sections these large clear cells with sharply formed spindles is much simpler than it may appear to those accustomed to less favorable material. The chromosomes were first outlined under the camera lucida

in their actual relation to each other in the sections, then a careful study was made and details filled in when necessary. Later, the chromosomes represented in plate 1 were arranged roughly according to size and placed on the plate so that the transverse rows represent the chromosomes found in one cell while the vertical rows represent the corresponding chromosomes in different cells. The eight complexes shown on this plate are typical of the conditions found in the hundred cells. They are so placed that the accessory is always passing to the upper pole.

Taking up first the small one at the right (no. 1), which is one of the heteromorphic pairs, we find in five of the cells (*b, c, d, f, h*) the atelomitic dyad going to the cell which lacks the accessory, while in three (*e, g, i*) it is going into the same cell as the accessory. The other two heteromorphic chromosomes, numbers 7 and 8, respectively, are so nearly identical in size and behavior that no attempt was made to distinguish between them. Instead, the segregation of their homologues in relation to each other and in relation to the accessory was noted and gave all of the information desired. If these two chromosomes are compared with each other in the first four cells (*b, c, d, e*), it will be observed that the dyads with nonterminal fiber attachment are going to opposite poles; in the remaining four cells (*f, g, h, i*) they are going to the same pole. But in cells *f* and *g* they approach the pole which lacks the accessory, while in the last two they will enter the same cell as the accessory. Now, if we compare chromosome number 1 with numbers 7 and 8, it will be seen that its dyads also segregate independently of either of the others. For instance, in the last four cells (*f, g, h, i*) its atelomitic dyad passes, either to the same second spermatocyte as the similar dyads of the larger chromosomes (*f* and *i*), or to the cell which receives the telomitic dyads (*g* and *h*). It is evident then that here are four chromosomes (nos. 1, 4, 7, 8) for which this is the segregation division and that they are distributed more or less without regard to each other or to the second spermatocytes.

If there is free segregation, the number of equally possible combinations in the gametes of a single individual is repre-

sented by the formula 2^n in which n represents the number of chromosomes in the reduced series; that is, the number of pairs of homologues. In this instance $n = 4$ since we are considering only the accessory and the three heteromorphic tetrads. Then 2^4 , or 16, is the possible number of combinations of these chromosomes in the gametes of this individual. While the number of morphologically different gametes formed as a result of the segregation of any *given* three is 8 (2^3), of any *given* two is 4 (2^2) of a *given* one 2 (2^1). The occurrence of *any* combination is shown by the coefficients of the expanded binomial raised to the n th power in which case n again represents the number of homologous pairs. In this instance the series of coefficients is 1-4-6-4-1.

From the two formulae given above we should expect to find

Any <i>given</i> 4 V's in $\frac{1}{16}$ of the gametes	Any 4 V's in $\frac{1}{16}$ of the gametes
Any <i>given</i> 3 V's in $\frac{1}{8}$ of the gametes	Any 3 V's in $\frac{1}{8}$ of the gametes
Any <i>given</i> 2 V's in $\frac{1}{4}$ of the gametes	Any 2 V's in $\frac{1}{4}$ of the gametes
Any <i>given</i> 1 V in $\frac{1}{2}$ of the gametes	Any 1 V in $\frac{1}{2}$ of the gametes
and 0 V in $\frac{1}{16}$ of the gametes	and 0 V in $\frac{1}{16}$ of the gametes

The difference—it will be noted—between these two series is in regard to *any* one, two or three as opposed to a *given* one, two or three; that is, in the latter case, we must distinguish between the V's with which we are dealing. A *given* V (as the accessory) would be found in one-half of the gametes, but, on the other hand, one-quarter of the gametes would contain only one V.

Perhaps both may be better shown graphically. Let A, B, C, D, represent the V-shaped homologues and the accessory and a, b, c, d, the rod-shaped homologues of the tetrads and the absence of any homologue in the case of the accessory. There are then the foregoing sixteen combinations any one of which is equally probable; *all* four V's together, e.g., A B C D, one-sixteenth of the time; *any* three, as in the second division, four-sixteenths of the time; *any given* three, e.g., A, B, C, two-sixteenths of the time; *any* two, as in the third division, six-sixteenths of the time; *any given* two, four-sixteenths of the time; while *any given* one occurs eight-sixteenths of the time and *only* one four-sixteenths of the time.

A B C D	a B C D	a b C D	a b c D	a b c d
	A b C D	A b c D	a b C d	
	A B c D	A B c d	a B c d	
	A B C d	a B c D	A b c d	
		a B C d		
		A b C d		

To be specific if A = accessory, B the V-shaped dyad of number 1 and C and D the indistinguishable ones of numbers 7 and 8, then, out of sixteen second spermatocytes, we should have the accessory in eight-sixteenths, the accessory and V of tetrad number 1 in four-sixteenths, any two V-shaped dyads six-sixteenths, any three in four-sixteenths. As the one hundred spermatocytes counted represent two hundred derivative cells, we have:

	Expected	Actual count
A given V (Accessory or no. 1)	$\frac{1}{2} \times 200 = 100$	100
Only one V.....	$\frac{1}{4} \times 200 = 50$	48
Two given V's (Accessory and no. 1 or those of 7 and 8)	$\frac{1}{4} \times 200 = 50$	46 and 47
Any two V's.....	$\frac{3}{8} \times 200 = 75$	84
Three given V's (nos. 1, 7, 8 or accessory, 7, 8)	$\frac{1}{8} \times 200 = 25$	22 and 21
Any three V's.....	$\frac{1}{4} \times 200 = 50$	48
All four V-shaped.....	$\frac{1}{16} \times 200 = 12\frac{1}{2}$	8

Considering that these figures are based on only one hundred first spermatocytes, the results are probably as near the expectations as could be anticipated for any objects on the basis of chance distribution for a like number of trials.

b. Recombination of the homologues of the J-shaped tetrads in eighty-two individuals of Trimerotropis (?) fallax (?). The use of the word recombination may require some explanation. The two dissimilar homologues composing a heteromorphic tetrad are segregated into different male gametes. For example the atelomitic dyad of a J-shaped tetrad goes to one pole and the telomitic dyad to the opposite pole. Gametes carrying each kind of homologue would therefore be formed. If we may assume that the form of the homologues remains constant and that a female may carry J-shaped (heteromorphic) tetrads and produce different types of gametes, in respect to such chromosomes, like those of the male; then, if free fertilization occurs, there should be found in the offspring all possible recombinations of the

homologues; e.g., tetrads with two atelomitic dyads and tetrads with two telomitic dyads as well as J-shaped tetrads. The only way to demonstrate that such recombination occurs is through breeding experiments. But since I have collected at random a considerable number of individuals (eighty-two) and have analyzed their first spermatocyte complexes, and have found the types of chromosomes which would be expected from the above assumptions, I have ventured to use the term recombination to express the relationship of those different types to each other.

It is obvious from the above section, that the two individuals there considered formed sixteen sorts of spermatozoa, so far as the selected elements are concerned. A study of a comparatively large number of animals was undertaken for the purpose of finding, in the first place, whether or not the complex is constant for the species and, secondly, if not, to determine the range of variation. The results from sixty-two individuals are given in plates 2 to 9. The chromosomes on these plates are arranged in the same manner as on plate 1, except that only one complex from an individual is shown; therefore each of the horizontal rows represents one animal. There are two exceptions to this statement: number 32a' is a spermatogonial metaphase from the same individual as number 32, and number 63a' is a spermatogonial metaphase from animal number 63.

It will be seen from plate 1 that animal number 1 has, as already stated, in addition to the three J-shaped tetrads, four atelomitic and four telomitic chromosomes. Such a complex was found to be constant for two individuals. This statement is based on one hundred camera lucida drawings. Only one complex is shown from the succeeding animals, but a comparative study of a number of complexes was made in each case so that it can be safely stated that the complex is practically constant in any one individual.

Taking up animal number 2 (plate 2), we find five atelomitic tetrads, numbers 7, 9, 10, 11 and 12; one heteromorphic tetrad, number 1; and the remaining five telomitic. Passing on to animal number 3 we find six atelomitic, three heteromorphic (J-shaped) and only two telomitic tetrads. Chromosome number 1

which in the preceding animals has been heteromorphic has both homologues of the atelomitic type. If we study individual number 6 we find that it has no J-shaped tetrads; it has eight atelomitic and only three telomitic tetrads. Comparing individuals 6 and 7 it is seen that chromosomes number 9 to 6 inclusive are atelomitic in the former and J-shaped in the latter.

It is also interesting to compare more in detail individuals number 2 and number 6. Chromosome number 1 in individual number 6 is the type which would be expected to result if the spermatozoon carrying the V-shaped dyad of this chromosome in animal number 2, for instance, united with an egg bearing a similarly shaped dyad. The shape of chromosomes number 6 and number 8 of animal number 6 may be accounted for on a similar assumption. Conversely we would expect to find, then, in some individual the two telomitic homologues of these two tetrads, and this is exactly what we have in animal number 2.

Clearly, then, the complex is not constant in this species, according to our past conceptions of constancy as indicated by form of chromosome, since a given chromosome in one member of this species may be of the *Stenobothrus* type, in another of the *Hippiscus* type, while in a third, one homologue of this same chromosome is of the *Stenobothrus* type and the other of the *Hippiscus* type. This is most easily demonstrated by following chromosome number 1 through several plates. Taking plates 2 and 3 we find this element of the mixed type in eight of the sixteen individuals, while it is of the *Stenobothrus* type in four and of the *Hippiscus* type in four. For the entire sixty-two individuals under consideration this tetrad is of the *Stenobothrus* type thirteen times, *Hippiscus* type eighteen times, one homologue of each type thirty-one times. This comes very close to the 1-2-1 ratio that we might expect on Mendelian principles if the two types occur with equal frequency in nature and chance fertilization occurs.

The next point was to determine, so far as my material permitted, the range of variation. I wish to emphasize the statement previously made that the arrangement in the plates ac-

according to size is only a rough estimate. Numbers 1, 2 and 4, I feel sure, are correctly identified in all cases, but aside from these there are scarcely any two consecutive ones which might not exchange places, yet the extremes are clearly distinguished. As the chromosomes are arranged, numbers 10, 11 and 12 are rings of the *Stenobothrus* type in all cases. I am inclined to think that this is correct. However, chromosome number 9 of the fifth animal might well change places with number 11. I have not been able to estimate satisfactorily the quantity of chromatin in the rings. On the other hand, it is readily seen on the slides that a given chromosome may be stretched out considerably with a comparatively slight thinning to compensate. The difference between an early and a late metaphase is also very striking. In the former the chromosomes are short and thick while in the latter they are evidently coming under some force which tends to stretch them out along the spindle axis. There is some evidence that this force is exerted through the spindle fibers; for instance, the accessory in individual number 45 (plate 7) has numerous fibers from both poles attached to it and its appearance certainly indicates that force is being exerted at the points of attachment.

It will be noted from the foregoing that any J-shaped tetrad has the capability of appearing in other individuals as a chromosome of either the *Stenobothrus* or *Hippiscus* type. Using this knowledge as a key, it is evident from a study of animals number 3 and number 32 (plates 2 and 5) that all of the chromosomes except number 2 and number 3 possess the potentiality of being of the *Stenobothrus* type in other individuals. If we then follow chromosomes number 2 and number 3 through the plates, we find chromosome number 3 in individual 16 (plate 3) to be a J, and in individual 21 (plate 4) we get the combination of two atelomitic homologues giving a *Stenobothrus* type of tetrad. Again, in individual number 31 (plate 5) it is a J. It is also of this form in animals number 7 (plate 2) and number 22 (plate 4). Chromosome number 2 does not occur except as a tetrad of the *Hippiscus* type in my collection. There was some fear that I might be confusing chromosomes number 3 and

number 5 because of their similar size and because not more than one in any individual varied from the *Hippiscus* form until I reached individual number 31 (plate 5). Here all of the chromosomes are either of the *Stenobothrus* type or J's except numbers 1 and 2 and they are too small for any confusion. Number 1 is freely of either type, so that we should expect to find, if a sufficient number of animals were studied, some with ten tetrads of the *Stenobothrus* type. The nearest approach to this possibility in these sixty-two specimens is the occurrence of eight in individual number 6 (plate 2). On the other extreme, it has already been noted that three rings and the accessory are atelomitic throughout. This would leave a possibility of eight telomitic tetrads. Individual number 62 (plate 9) is of this composition.

Plates 2 to 5 present quite a different appearance from plates 6 to 9, for the former group has many more chromosomes with nonterminal fiber attachment than the latter. The results are recorded in the order in which the animals were collected, which was also the order in which they were studied. The individuals represented on plates 2 to 5 were taken in the Yosemite Valley; Sisson, Cal.; and Eugene, Ore. Those represented on plates 6 to 9 are from Friday Harbor and Olga, Wash.; La Grande, Ore.; and Pocatello, Ida. They grouped themselves as shown without the slightest rearrangement. I refer to the first group as form A and to the second as form B.

The following table, made up from the plates, gives the total number of atelomitic and J-shaped tetrads. The first and third columns represent form A and the second and fourth columns represent form B. Each horizontal row, therefore, represents two individuals. The first horizontal row represents individual number 2 (plate 2), form A, in columns 1 and 3, and individual number 33 (plate 6), form B, in columns 2 and 4. The successive horizontal rows represent successive individuals in the two forms, i.e., numbers 3 form A, and 34 form B in the second row, numbers 4 form A, and 35 form B, in the third row, etc.

The ratio of atelomitic to J-shaped tetrads in the case of form A is 0.42; in the case of form B, 0.47. One might have expected in place of this relatively constant relation that the larger num-

NUMBER ATELOMITIC TETRAIDS	NUMBER ATELOMITIC TETRAIDS	NUMBER OF J TETRAIDS	NUMBER OF J TETRAIDS
<i>Form A</i>	<i>Form B</i>	<i>Form A</i>	<i>Form B</i>
5	4	1	1
6	4	3	2
5	4	4	2
5	3	3	1
8	3	0	1
4	4	5	1
5	3	2	2
7	3	2	2
5	4	2	1
6	4	1	1
4	3	4	2
5	4	1	2
4	4	4	2
6	4	1	1
6	5	2	2
6	3	2	4
6	4	3	2
3	3	5	1
6	3	2	2
7	4	1	3
7	4	2	2
5	3	3	1
5	4	3	2
4	4	3	1
6	3	1	3
6	4	2	1
3	3	4	2
6	3	1	5
7	4	1	1
6	3	3	6
5	3	4	1
Average... 5.77	3.6	2.4	1.7

ber of *Stenobothrus* rings in form A would be compensated for by a smaller number of J's, or heteromorphic forms and the smaller number of *Stenobothrus* rings in form B by a larger number of J's. This might have indicated that one form was more viable in a given environment than the other. What we have is a uniform decrease in both *Stenobothrus* rings and J's in form B and a compensatory increase in the number of telomitic tetrads. The most important point to be noted is that if we may assume that heteromorphism is an indication of heterozygosity, then form

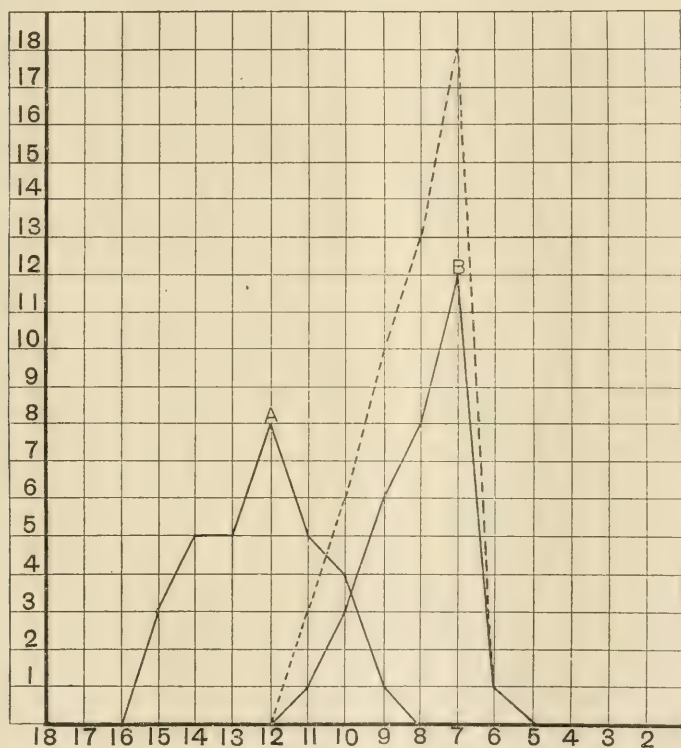
B is only about one-half as heterozygous as form A and consequently may be expected to be less variable taxonomically. That it really is less variable is indicated by the fact that taxonomists have separated the individuals that largely compose form A into four species, while they have treated the group that chiefly composes form B as a single species.

Since chromosome number 1 is the only tetrad subject to heteromorphism which could be recognized without possibility of confusion, its composition was studied in the two groups. Its behavior in the whole collection has been described on page 458. It occurs as follows:

	ATELOMITIC	J-SHAPED	TELOMITIC
Form A	8	16	7
Form B	6	16	9

In both cases it approximates closely a 1-2-1 ratio as it did for the entire collection. While it is evident then, that a rather general change has taken place in the two forms in respect to the number of atelomitic and telomitic chromosomes, it has not affected chromosome number 1. Likewise, a glance through the plates will show that the accessory (no. 4) and chromosome number 2 have remained constant. The remaining nine tetrads intergrade in size so closely that it is impossible to identify any given one with certainty. It was, therefore, necessary to study them collectively. The number of atelomitic dyads per individual was determined and the results plotted. The curves thus derived are shown in text figure 1. Numbers 10, 11 and 12 have remained constantly atelomitic, if I have been correct in my identification of them. But on the chance that I may not have been able to identify these correctly I shall include them in the curves; if their form is constant, they will not affect the shape of the graphs, while, on the other hand, there is a possibility that they are concerned in the changes. In text figure 1 the ordinates represent the number of animals; the abscissae the number of atelomitic dyads per cell. That is, each *Stenobothrus* ring is made up of a pair of atelomitic dyads, while the J-shaped tetrads have only

one. For example, individual number 3 has five *Stenobothrus* rings and three J-shaped tetrads making thirteen V-shaped dyads derived from the tetrads in question—that is, not including numbers 4, 2 or 1. We have then in these nine (fig. 1) tetrads, which



Text-fig. 1 Curves showing the number of atelomitic dyads, exclusive of chromosomes number 1, 2 and 4. The abscissae represent the number of such dyads, the ordinates the number of individuals, the broken curve includes data from twenty additional animals of form 'B.'

are concerned in the changes, the possibility of eighteen atelomitic dyads. The two solid curves each represent the same number of individuals, thirty-one, the total number in the collection of form 'A'. There were twenty more animals of form 'B' which have been studied but not included in the plates, since

they show no exceptional conditions. Data from these twenty are used in the broken curve of form B, which includes all of the specimens of this form in my collection.

Analysing these curves we see that in form A the extreme numbers of atelomitic dyads are 15 and 9, the mode 12 and the mean 12.3. In form B the extremes are 11 and 6, mode 7 and mean 8. It will be noted that the extremes in neither group reach to the mode of the other. The graph of form A is well balanced, indicating a stable group with considerable range of variation. That of form B, while showing much less variation, is skewed to the left, which would lead one to suspect that this group is changing towards the more stable form.

Plates 6 to 9 show chromosomes number 3 and number 5 always of the Hippiscus type in form B, though both are subject to change in form A. There is some question as to whether chromosome number 5 is always correctly identified. However, it is clear from animal number 60, which has three rings of the Stenobothrus type and five J-shaped tetrads, that eight tetrads have the potentiality of being atelomitic. It is puzzling that five (animal no. 47) is the nearest approach to this extreme found. Animal number 60 with its possibility for eight Stenobothrus rings offers one of the strongest indications that these two groups may be really members of a single species.

c. Segregation and recombination of homologues of J-shaped tetrads in eleven individuals of Circotettix lobatus. Eleven specimens of this species were taken in a very circumscribed area at La Grande, Ore. and a single specimen of another species (rabula) at Ogden, Utah. This latter animal had both telomitic and atelomitic chromosomes, but no J-shaped tetrads. This is not significant, however, as three of the eleven individuals of lobatus also lack J-shaped tetrads.

First spermatocyte complexes of eight of the eleven lobatus are shown (plate 10). The other three are included in the description. As just stated, three (or over one-fourth) of these animals have no J-shaped tetrads. Four have one and the remaining four have two. As in Trimerotropis, their dyads segregate at random in regard to each other and to the accessory.

The minimum of atelomitic tetrads in this limited collection

is four (animal no. 64, plate 10) and the maximum six (animals no. 69 and no. 71). Animal number 67 has, in addition to five telomitic tetrads, two J-shaped tetrads, exclusive of chromosome number 1, which in this instance is rod shaped, but which we see from other individuals may be atelomitic. In a large group of animals, then, one would expect to find a maximum of eight atelomitic tetrads. This species agrees in this respect with form 'B' described above. Chromosome number 1 is of especial interest; in four cases, it is of the *Stenobothrus* type, in three, rod-shaped, and in three, J-shaped, while in one animal (no. 70) it is extremely unequal. I will have more to say later concerning this point. Segregation and recombination of the dyads of the heteromorphic tetrads in this species does not differ from that in the larger group.

d. Origin of a tetrad with unequal dyads. Tetrad number 1, animal number 70, as mentioned above, divides very unequally as shown on plate 10. Furthermore, the fiber attachment is not terminal on the smaller part, which in size suggested at once that it was the free arm of the atelomitic dyad of the heteromorphic form of this tetrad. A study of the late prophase gave the key to the solution of the problem as to what might cause such an inequality. Here we find a tetrad, normal to all appearances, except that a pair of chromomere vesicles,² (so called plasmasomes) described by the writer in 1913, is attached near the middle of one dyad (fig. 70*d*, plate 11). Wenrich ('16) identified

² It seems desirable to qualify the term vesicle which I used ('13) to designate these structures as chromomere vesicles or vesicular chromomeres, as the case may be, in order to prevent confusion with the vesicular chromosome or chromosomal vesicles first described for the Orthoptera by Sutton ('00).

The suggestion comes that they may stand in somewhat the same relation to certain chromomeres as the chromosomal vesicles do to the chromosomes. Just as given chromosomes (e.g., the accessory) have a more marked tendency than others to become vesicular, these specialized chromomeres of different chromosomes may vary in this power. For example a chromomere, or granule, may merely become expanded with the chromatin more or less equally distributed as Wenrich ('16) has shown for one of the terminal granules of tetrad 'A' of *Phrynotettix*. Such an expanded chromomere may or may not go a step further and form a definite membrane giving a true vesicle; e.g., one of the terminal chromomeres of tetrad 'B' in *Phrynotettix*.

these vesicles as expanded conditions of certain granules, terminal in the case of his tetrads 'A' and 'B'. We also know from the work of Pinney ('09) that the point of fiber attachment is usually marked by prominent granules. It, therefore, seems probable, especially since the other end lacks them, that the vesicles mark the point of fiber attachment on what should have been a J-shaped tetrad and that their formation has weakened the dyad with which they are associated to such an extent that division in metaphase occurs at this point. The result is that one and a half dyads go to one pole, the remaining half dyad to the other. This peculiarity seems to be constant for this animal. Figure 38a (plate 11) represents a similar possible origin for an unequal pair in one of the larger chromosomes in form B. This was the only instance of the kind found in this animal.

e. Other forms of heteromorphism. Two other types of heteromorphism remain to be considered. The first—a peculiarity common to both these genera—is a constriction of certain dyads of chromosomes number 3 and number 5. The behavior of tetrad number 5 in different individuals is shown in plate 14. Figures 21a and 13a are homomorphic, constricted and smooth forms, respectively. Figure 62f is the heteromorphic form. The same thing is shown in chromosome number 3 (plate 2), animal number 6 being homomorphic for the constricted form, number 2 homomorphic for the smooth rod and number 8 heteromorphic. Tetrads marked by this peculiarity are almost as striking as the J-shaped tetrads. And since chromosome number 5, frequently, and number 3, occasionally, are J-shaped or even of the *Stenobothrus* type, the J-shaped tetrads may have the telomitic dyads of the constricted form (plate 3, 16, chromosome no. 3); or of the usual smooth rod form. In other words, for these two chromosomes, there are three types of homologues: atelomitic, telomitic-smooth and telomitic-constricted.

The third type of heteromorphism affects chromosomes number 9 and number 11 as they are arranged on the plates. Both homologues are atelomitic, but there has been a secondary shifting of the point of fiber attachment so that the free arm of one dyad is much longer than the free arm of the other. The point

of union of the homologues is correspondingly nearer the bend in the dyad with the long free arm. Photomicrographs of these conditions are shown, figures 22*c* and 22*d* (plate 14). As indicated by the numbers, these two chromosomes occur in the same individual (plate 4, 22). They are not modifications of the same chromosome, for in numerous instances both are present in a single section of a cell. Figure 23*a*, from another specimen, shows a tetrad which is homomorphic for the long-armed condition of the homologous chromosome shown in figure 22*c*, while figure 10*a* is probably the homomorphic short-armed form of the same chromosome from another specimen. At least, this latter individual is homomorphic for the short-arms as may be seen from the drawing of the entire complex (plate 3, 10, chromosome no. 11). The homomorphic forms of tetrad number 9, shown in 22*d*, are represented in figures 17*a* and 10*b* (plate 14). Chromosome number 9 may also be J-shaped (plate 2, 7) or telomitic (plate 4, 25).

All possible combinations of the dyads in these two types of heteromorphic tetrads occur and segregate freely in relation to sex.

In the two heteromorphic types described above, as in the case of chromosome 'C' in *Phrynotettix* (Wenrich '16), we have a visible mechanism whose behavior in the maturation divisions corresponds to the segregation of triple allelomorphs.

f. Reduced number of chromosomes in Circotettix. *Circotettix* is the only genus of the Oedipodinae, so far investigated, to have less than the typical number of chromosomes. Numerous counts showed eleven to be the number in the first spermatocyte and twenty-one in the spermatogonia (plate 11, figs. 70*a* and 75). I am not satisfied as to whether the missing chromosome is number 2 or number 3. Plate 10 is arranged to emphasize its absence. The figures are from *Circotettix lobatus*, but *rabula* agrees in this respect. It is idle at present to speculate as to whether the chromatin which would have gone to form the missing tetrad has disappeared from the complex or whether it has united with some other tetrad to form an octad such as Robertson ('16) postulates to account for the reduced number in

Stenobothrus. This latter genus has but nine chromosomes in the reduced series (seventeen in spermatogonia). Three of these, however, are rings which present a typically different appearance from the remaining five tetrads. The basis of this difference is that they have entered the spindle extended parallel to its axis and have nonterminal fiber attachment. Robertson holds that these three chromosomes are really multiples; their point of union being represented by the position of the spindle fiber attachment. This would give twelve (the number typical for the *Acerididae*) as the real number of chromosomes in the haploid series. Woolsey ('15) has clearly shown such a linkage of chromosomes to be correlated with a reduction in number in several species of a *Locustid* genus. Furthermore, McClung ('16) has found conditions similar to those described by Miss Woolsey among the individuals of a single species of *Hesperotettix*.

The difficulty is, that Robertson puts forward a hypothesis that nearly all rings of the *Stenobothrus* type are multiples and are correlated with a reduction in the number of chromosomes. This is clearly not the case as regards either *Trimerotropis* (forms A and B) or *Circotettix*, for both have numerous tetrads of the *Stenobothrus* type and *Trimerotropis* has the normal number of chromosomes (twelve) while *Circotettix* has but one less. The number of rings of the *Stenobothrus* type varies from individual to individual, but the number of chromosomes is constant. In addition, the transition stage (J-shaped tetrad) between chromosomes of the *Stenobothrus* type and those of the *Hippiscus* type is present in these species. A further argument against the above hypothesis is the secondary shifting of the point of fiber attachment on certain chromosomes (figs. 22c and 22d, plate 14).

It is clear that we have two fundamentally different types of rings which are so similar morphologically that unless we get transition stages, as Woolsey ('15) has in *Jamaicana* and McClung ('16) in *Hesperotettix*, we have no criterion by which to separate them. Both enter the metaphase extended parallel with the axis of the spindle, both have non-terminal spindle fiber attachment, both give double V's in anaphase and both may occur where there is reduction in number without all of them being

correlated with the reduction (Circotettix and the Stenobothrus-like form described by McClung, '14). Yet structurally one is an octad the other a tetrad. The octad has true terminal spindle fiber attachment, so far as the tetrads composing it are concerned, while the ring of the other type has real nonterminal spindle fiber attachment.

g. Supernumeraries. Another variation which has attracted attention in our collection of Acrididian material for the first time and which is relatively very abundant in the specimens collected during the summer of 1915, is the presence in certain individuals of one, or sometimes two, entities which I shall designate as supernumeraries. They possess the staining capacity of chromatin. In the spermatogonia they enter the spindle at metaphase and divide like any chromosome (fig. 62*a*, chromosome *s*, plate 11 shows this body in the spermatogonia). In the plates of the first spermatocytes these bodies have been placed to the left of chromosome number 12, and a glance through plates 2 to 10 will show their frequency of occurrence. Individual number 10 (plate 2) of form 'A' possessed two supernumerary chromosomes, one more than twice the size of the other. This was the only animal in this group which possessed supernumeraries. But when we pass to form 'B' (plates 6-9) we find them in eight out of thirty-one individuals, that is, in more than one-fourth of all the animals studied.

I would especially call attention to the similarity between the supernumerary and accessory in specimen number 62.

The supernumeraries are also present in two of the eleven Circotettix studied. It is evident from the plates that these elements vary greatly in size; when two are present in a given individual there may be very little resemblance between them. At the first spermatocyte division they pass to one pole undivided. When two are present they segregate freely in relation to the accessory and to each other. At the second spermatocyte division they again enter the spindle, at least usually, and divide as in the spermatogonia (plate 13, 62*e*).

2. Spermatogonial metaphases and female somatic metaphases

a. *Individual number 1.* Dr. McClung ('14) has shown that generally the point of spindle fiber attachment is constant in all generations (spermatogonia, first and second spermatocytes). Therefore, knowing the number of dyads in the first spermatocyte with nonterminal fiber attachment, one would expect to find the same number in the spermatogonia. The animal represented on plate 1 has four *Stenobothrus* rings, each made up of a pair of dyads with nonterminal fiber attachment or eight such dyads in all; in addition, there are the atelomitic dyads of the three J-shaped tetrads and the accessory; so that we should expect twelve atelomitic chromosomes in the spermatogonia of this animal. Figure 1, plate 11, a spermatogonial complex from this specimen, shows the twelve atelomitic chromosomes. Entire complexes of spermatogonia were drawn in order to be certain that all of the chromosomes with nonterminal fiber attachment might be present. The telomitic chromosomes appear in outline only, as I wish to make the enumeration of the others as easy as possible.

b. *Extremes found in the group.* In order to show clearly the correspondence between the spermatogonial chromosomes and those of the first spermatocytes, two spermatogonial complexes, one from Form 'A', the other from Form 'B', have been rearranged so that the homologues are paired as they are in the first spermatocyte anaphase (with which they are compared) from the same individual. One of these spermatogonial complexes is shown with the chromosomes in their normal position in figure 32a (plate 11). The rearrangement of this complex may be seen on plate 5, figure 32a. Fifteen atelomitic dyads are present in both spermatogonium and first spermatocyte. Figures 36 and 63a' (plate 9) show similar arrangements of first spermatocyte anaphase and spermatogonial complex, respectively. Each has eight atelomitic chromosomes. Figure 63a' is a rearrangement of 63a (plate 11).

From the analysis of the first spermatocyte metaphases, we would expect animal number 62 to have the least number of atelomitic spermatogonial chromosomes, seven. This is seen

to be the case in figure 62a (plate 11). Information derived from the same source would lead us to expect seventeen atelomitic chromosomes in individual number 22. Such a metaphase is shown on plate 11, figure 22a. Figures 62a and 62b are spermatogonial metaphases from the same animal and have seven atelomitic chromosomes, as we would expect from the first spermatocyte complex of this individual (plate 9, 62).

Figures 70a and 75 (plate 11) are spermatogonial complexes from *Circotettix lobatus*. The significant difference from similar complexes of *Trimerotropis* is that they have twenty-one instead of twenty-three chromosomes. In regard to the atelomitic members the conditions are the same as in *Trimerotropis*, that is, the number of such dyads is the same in both spermatogonial and first spermatocyte metaphases. The number is twelve in these two cases. Figure 70a is from the individual in which tetrad number 1 divides very unequally in the first spermatocyte. It will be seen from this figure that the smallest two homologues are equal in size but one has median a fiber attachment. This agrees with the expectations from the study of the prophases of the first spermatocyte which showed a normal tetrad. We have then in the tetrad which divides unequally in the first spermatocyte, in reality a J-shaped tetrad which is dividing at the point of fiber attachment in the atelomitic dyad.

c. Female somatic complexes. Unfortunately, the maturation stages in the female are inaccessible because they do not take place until a few hours before or even after the egg is deposited (Henking '91), and the great quantity of yolk present has so far defied all efforts at sectioning. Immature ovaries are readily sectioned and contain numerous divisions in the follicular cells. These have been used for the somatic complexes. Morrill ('10) found from a study of the cleavage and early blastoderm stages of some Coreid Hemiptera that the number and size relations of the chromosomes in the somatic cells of the males and females are the same as in the spermatogonia and oogonia, respectively. Three females were studied by me, the complex proving constant for the individual, but

varying from animal to animal. Specimen number 74 with its fourteen atelomitic chromosomes (plate 11, 74) was taken in the Yosemite Valley, hence should belong to the group having the larger number. The other two are from Puget Sound, where we would expect the smaller number. One has ten (plate 11, 72) and the other, much to my surprise, has eight (plate 11, 73), which, if my conclusion that three of the atelomitic rings are constant is correct, would be the minimum number for the female, since she possesses two accessories, giving a complex of twenty-four chromosomes instead of twenty-three as in the male.

We may now answer the third and fourth questions propounded at the beginning of this paper. The complex is constant for any given individual but varies within fixed limits for the species. Hence there is no need to assume any regulatory mechanism during maturation or fertilization.

3. *Second spermatocytes*

a. Types found in individual number 1. As has already been noted, the formula, 2^n , in which n represents the number of pairs of chromosomes under consideration, gives the possible combinations in the gametes. According to this formula animal number 1, as we have seen on page 455, would be expected to form sixteen sorts of second spermatocytes. However, as we can not distinguish between chromosomes 7 and 8, we can identify only twelve sorts, as shown on plate 12. Numerically, they fall into two classes, those with eleven (figs. 1 *j* to *o*) and those with twelve chromosomes (figs. *p* to *u*), owing to the presence or absence of the accessory. In the class with eleven chromosomes we would always have the four atelomitic chromosomes derived from the four *Stenobothrus* rings (fig. 1 *j*). In addition we may have one of the larger atelomitic dyads (fig. *k*), the small atelomitic dyad (fig. *l*), the small atelomitic dyad and one of the large ones (fig. *m*), both large ones without the small (fig. *n*), or the atelomitic dyads of all three (fig. *o*). Figures *p* to *u* present a similar series, except that they each have one more atelomitic dyad, the accessory.

b. Extremes found in the group. Two individuals, number 6 (plate 2) and number 62 (plate 9), have no J-shaped tetrads and therefore would have only the two recognizably different sorts of second spermatocytes determined by the presence or absence of the accessory. Number 7 (plate 2) and 60 (plate 9), on the other hand, each have five J-shaped tetrads which, in combination with the accessory, would give 2^6 or sixty-four different sorts of spermatozoa. The distribution of the J-shaped tetrads in forms 'A' and 'B' as well as in the whole group, is shown by the curves (text-fig. 2). The ordinates represent number of individuals, the abscissae the classes of spermatozoa formed. The lower broken curve represents form 'A', the solid line form 'B', while the upper broken line is a composite of the two. It is interesting to note that while the extremes of both forms are the same and contain the same number of animals, there are only four of the thirty-one members of form 'B' which give more than eight kinds of spermatozoa, while fifteen, or practically half, of the members of form 'A' give sixteen or more kinds.

Individual number 62 is of interest on account of the large supernumerary present in the spermatogonia and first spermatocytes. There are no J-shaped tetrads in this specimen, but it is possible to recognize four sorts of second spermatocytes. Of two sorts with three atelomitic dyads one has eleven chromosomes and the other twelve—due to the presence of the supernumerary. Two sorts have four atelomitic dyads, i. e., the accessory in addition to the three derived from the three *Stenobothrus* rings of the first spermatocyte. One of these has the normal number (twelve) and the other thirteen, since the latter also contains the supernumerary.

Individual number 63 (plate 9) is one of the easiest to follow out, since it has only one J-shaped tetrad. This, with the accessory, gives two differential chromosomes, from the distribution of which we should expect 2^2 or four sorts of second spermatocytes. Considering all the atelomitic dyads, including those derived from the three *Stenobothrus* rings, we find four

morphologically different sorts of second spermatocytes as follows:

- 11 with 3 atelomitic dyads (fig. 63*d*)
- 11 with 4 atelomitic dyads (fig. 63*c*)
- 12 with 4 atelomitic dyads (fig. 63*b*)
- 12 with 5 atelomitic dyads (fig. 63*e*)

The transformation stages which result in the mature spermatozoa appear to be perfectly normal throughout the collection.

c. Circotettix. *Circotettix* does not differ from the species just described so far as the formation of different types of spermatids is concerned. But we have the important difference that the numbers are ten and eleven (barring the supernumeraries which are present in two of the specimens) instead of the usual eleven and twelve. Complexes from two individuals are shown in figures 69*a*, 69*b* and 70*b*, 70*c* (plate 13).

III. SUMMARY OF OBSERVATIONS

1. The number of spermatogonial chromosomes in *Trimerotropis* is twenty-three. Anywhere from seven to seventeen of these have been found to be atelomitic, but the number of such chromosomes is constant in a given individual.

2. The number of spermatogonial chromosomes in *Circotettix* is twenty-one. Nine to thirteen of these have been found to be atelomitic in different individuals. The number of atelomitic chromosomes is constant for the individual.

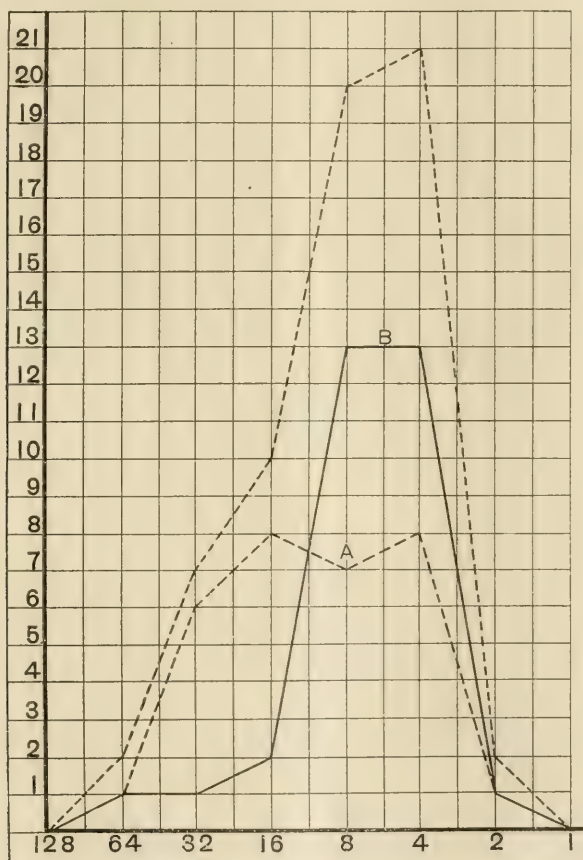
3. The number of somatic chromosomes in the female of *Trimerotropis* is twenty-four. The number of atelomitic chromosomes varies in different individuals from eight to fifteen, but is constant for the individual.

4. The number of chromosomes in the first spermatocyte of *Trimerotropis fallax* is twelve, of *Circotettix* eleven.

5. Certain individuals may possess one or two supernumerary chromosomes. These bodies divide in the spermatogonia, pass to one pole undivided in the first spermatocyte division and divide in the second spermatocyte. They segregate in the

first spermatocyte without relation to the accessory, or to each other when two are present.

6. The first spermatocyte tetrads show three types of heteromorphism: 1) one homologue telomitic, the other atelomitic,



Text-fig. 2 Curves showing the number of morphologically different classes of spermatozoa. The abscissae represent number of classes, the ordinates number of individuals. The large broken line is a composite of 'A' and 'B.'

giving J-shaped tetrads. 2) both homologues atelomitic, one with nearly median, the other with subterminal fiber attachment. 3) both homologues telomitic, one smooth, the other constricted; or, one telomitic constricted, the other atelomitic.

7. All of these types of heteromorphic homologues segregate at random in the first maturation division.

8. Considering the J-shaped tetrads alone, of sixty-two males, two formed sixty-four morphologically different sorts of spermatozoa, seven formed thirty-two sorts, ten formed sixteen, twenty formed eight, twenty-one formed four and only two were dimorphic.

9. The complex is constant in the individual for somatic, spermatogonial and first spermatocyte metaphases and has a fixed range of variation for the second spermatocytes. Certain exceptions to this general rule have been noted in the text, e.g., the accessory with numerous fibers attached (plate 7, 45) and the tetrad which divides unequally in *Circotettix* (plate 10, 70).

10. The complex varies widely in the group within the limits of the above mentioned types of heteromorphism, indicating, so far as my material goes, that random mating of the various classes of morphologically different gametes has occurred.

11. Taxonomically the group is exceedingly variable.

IV. DISCUSSION

1. Constancy in individuals

To me one of the most impressive facts in this whole work has been the very great degree of individual constancy of the complex which has been preserved in the organism in spite of the wide range of morphological variation between the homologous chromosomes in these species. It should be emphasized that the term individual constancy is applicable here, although it is used in a broader sense than is ordinarily the case; that is, while the somatic, spermatogonial and first spermatocyte complexes are practically identical for the respective generations in a given individual, there is a fixed number of morphologically different classes of second spermatocytes for each individual, but in all cases these can be calculated from a knowledge of the first spermatocyte complex.

It is futile to speculate at present on the cause of this heterogeneity, but so far as the doctrine of chromosome individuality

is concerned the manner of its origin is of minor importance. The essential thing is that it exists, and is transmitted, not only from cell generation to cell generation in the individual, but occurs so definitely that one can predict the combinations that should occur in the population. Judging from the conditions present in my material one would find all possible associations if a reasonably large number of specimens were examined.

Actual observation has shown the ratios of the telomitic to the atelomitic dyads of chromosome number 1 to be 1 to 1. In other words the variant, atelomitic homologue, occurs in practically one-half of the population. But we cannot generalize that this ratio holds, therefore, for all of the variants, if we may so designate all atelomitic dyads. Tetrad number three has only six atelomitic dyads out of a possible one hundred and twenty-four in the sixty-two individuals represented (plates 2 to 9). It is impossible to calculate ratios for the occurrence of any given combination in the general population unless one knows in advance the frequency of occurrence of that particular variation.

It is hard to imagine a better example of persistent individuality than that illustrated in figures 21*a*, 62*f*, 13*a*, plate 14 where a variation from the normal smooth rod form (fig. 13*a*), such as the constriction of the dyads (fig. 21*a*), has been transmitted, not only from cell to cell in one individual, but apparently on to the next generation, so that the union of a gamete carrying such a chromosome with one bearing its normal homologue has resulted in a zygote which shows both types clearly (fig. 62*f*). This is the same principle found throughout this work where no kind of heteromorphism (J-shaped tetrads and atelomitic tetrads with unequal arms) has been found without homomorphic forms corresponding to both types of dyads occurring in other individuals.

There are but two other instances in all cytological literature so far as I know where homologous chromosomes show morphological differences other than those of size. One of these is the multiple of *Mermeria* (McClung '05). The other case is that reported by Voïnov ('14). It seems desirable to undertake a

rather extensive review of the latter paper on account of some questionable conclusions and on account of some points of general similarity, as I believe, in our material.

Voïnov is dealing with *Gryllotalpa vulgaris*. This is the form for which vom Rath ('92) reported the somatic number of chromosomes as twelve. He described a doubling of this number just before the first maturation division which reestablished the normal number. The second division brought about reduction to one-half the normal. Vom Rath extended to all animals his conclusion that there is a doubling of the number of chromosomes before the maturation divisions, both of which are reductional and result in halving the normal number of chromosomes. As this erroneous idea appealed to many as a support of Weismann's hypothesis it was widely accepted for a time. Hence work on this form is of more than usual interest.

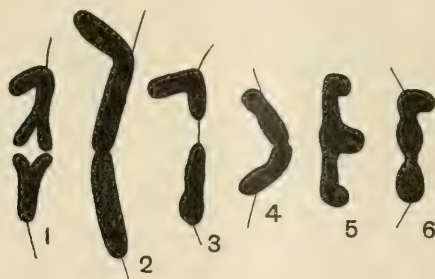
Voïnov figures fifteen chromosomes in the spermatogonia. The smallest of these is clearly without a morphological mate and he considers it to be in reality a pair of microchromosomes synapsed in the spermatogonia. This assumption leaves him without a *Y* for what he considers an *XY* pair which occurs in the first spermatocytes. He assumes the *Y* exists in the spermatogonia but has escaped detection. Therefore, he concludes that seventeen is really the somatic number, while he finds seven in both maturation divisions. This is accounted for by further assuming that two of the seven must be multiples; one an octad, the other a hexad. The hexad he believes to be identical in composition with the chromosome 'en L' of de Sinéty ('01) and the hexad of McClung ('05). A comparison of Voïnov's chromosome marked 'a' and 'bi' (text fig. 8c, see my text fig. 3, 3) with de Sinéty's chromosome *c-s* (fig. 93, plate 3, see my text fig. 3, 1) and with McClung's chromosome 'ac' (text fig. 3, see my text fig. 3, 2) will show a critical difference. In both the latter, if we may assume that de Sinéty's chromosome 'en L' is a hexad, the tetrad part divides, resulting in a greater quantity of chromatin passing to one pole than the other, while Voïnov's 'hexad' divides in such a manner that, though the parts are morphologically different, the quantity of chromatin in each

would seem to be about the same. Voïnov recognizes this difference, he says (p. 474):

Il s'ensuit que le chromosome accessoire est distribué seul à un pôle et le bivalent entier avec lequel il était associé va au pôle opposé.

Ce bivalent donc, comme le chromosome accessoire, ne se divise pas dans la première mitose de maturation. Cette manière de se comporter constitue une différence avec les chromosomes multiples décrits par Sinéty et Clung.

That is, while its structure is the same, its behavior is different. It will be obvious to the reader that this chromosome



Text-fig. 3 1 'Chromosome en L,' de Sinéty, plate III, figure 93.

2 Hexad multiple, McClung, '05, p. 309, figure 3.

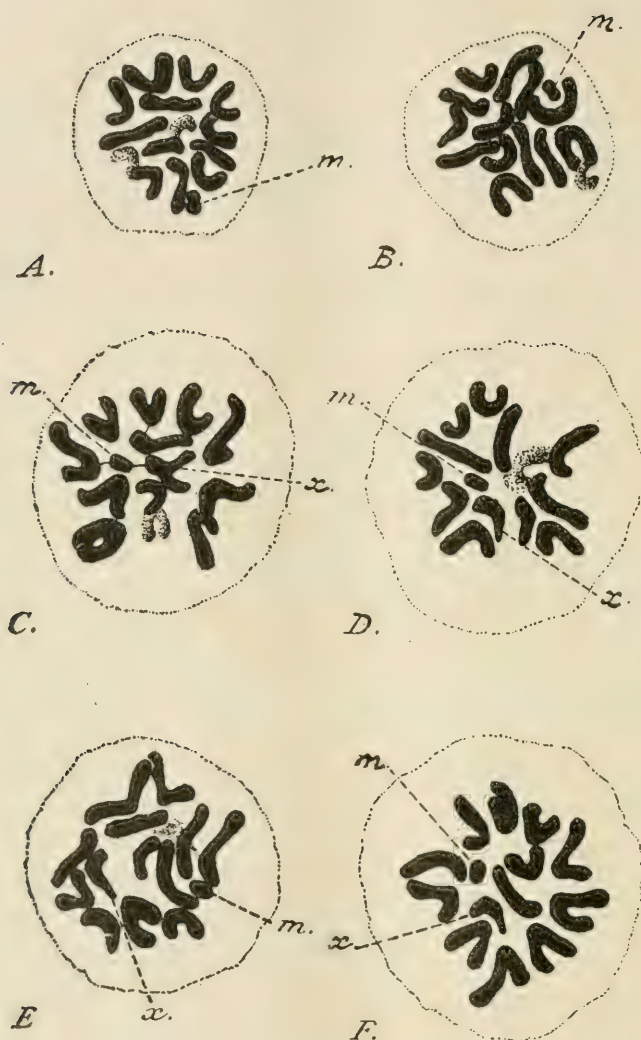
3 'Chromosome -L,' Voïnov, '14, p. 473, figure 8c.

4 Late second spermatocyte metaphase of 'l'element bivalent' Voïnov, p. 488, figure 16c.

5 'La grande tétrade,' Voïnov, plate XXIV, figure 32t.

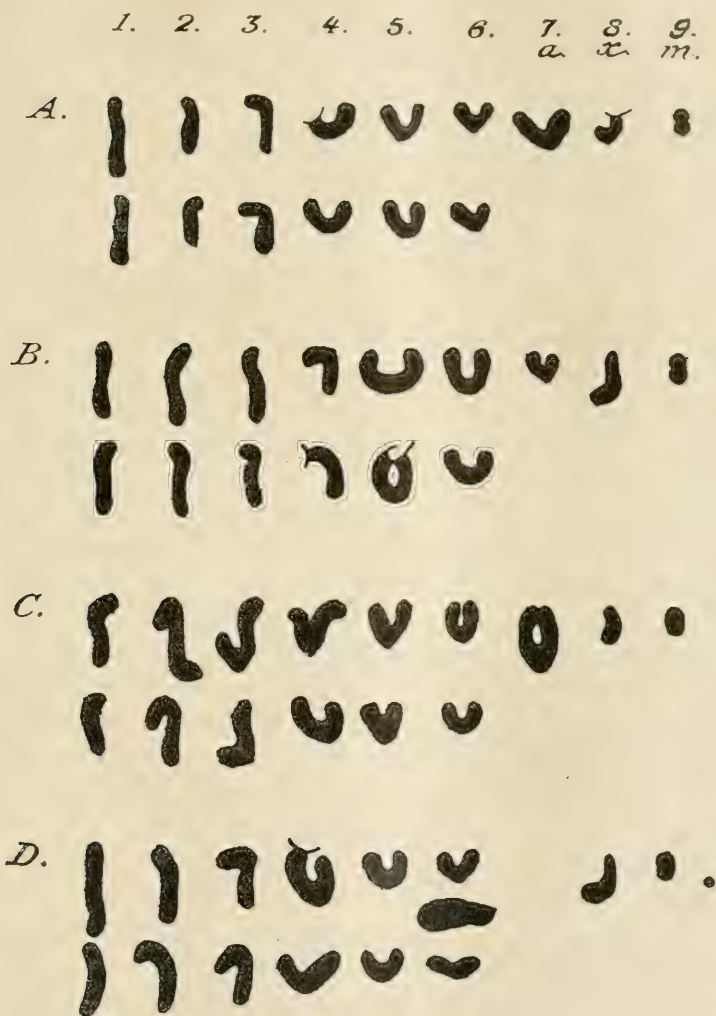
6 'L'idiochromosome, XY,' Voïnov, Plate XXIV, figure 29.

corresponds both in form and in behavior in the individual with the J-shaped tetrads described in this paper. But, if it does correspond, one would expect certain individuals to contain in its place a rod-shaped tetrad and others a ring of the Stenobothrus type. That such a ring occurs in Voïnov's material may be seen in his figure 32t, plate 24, or my text figure 3, 5. While he has material from fifty animals, it is impossible to judge how thoroughly it has been studied from the standpoint of the occurrence or non-occurrence of this 'hexad.' The only direct statement is in regard to a count to show the distribution of its parts in relation to those of an unequal pair. This



Text-fig. 4 A photographic reproduction of Voinov's text-figure 1, showing that different individuals possess different numbers of V-shaped (atelomitic) chromosomes. A and B from the same individual show eight or nine. C from a different animal at least eleven, possibly thirteen. D from a third animal nine or ten. E and F from a fourth specimen have eleven.

count, he states, is based on a single individual. However, if my interpretation is correct, one should find varying numbers of atelomitic chromosomes in the spermatogonia of different individuals, while on Vořnov's statement the complex should



Text-fig. 5 A photographic reproduction of Vořnov's text-figure 2. The difference in the number of telomitic and atelomitic chromosomes possessed by individuals B and C is well shown.

be morphologically the same for all individuals. Voïnov shows (p. 448, fig. 1, see my text fig. 4) spermatogonial complexes from four individuals. *A* and *B* are from the same individual and agree in having eight or nine atelomitic chromosomes. *D*, from another individual, seems to be of the same type, while *C* and *F* from two other individuals, have eleven or twelve. This variation is made conspicuous by the arrangement in his text-figure 2 which shows strikingly the difference of the complex of individual *C* from that of *B*. However, Voïnov states (p. 450) that the form and size are constant for the species. To quote: "Dans les dessins où les chromosomes des quatre premières plaques sont groupés suivant leur ressemblance (fig. 2), on voit que la forme et la grandeur sont constantes pour l'espèce." On the contrary, should one judge by these figures, (see my text-fig. 5) one would conclude that even the number was not constant since *D* lacks chromosome number 7. Also numbers 7A and 7B which purport to be homologous chromosomes,—drawn even from the same individual,—certainly bear no resemblance in size. This may better be attributed to the fault of the observer than to the material, as chromosome 7B would seem from the drawing to be much more homologous with 6A, and one member of 6B with 7A.

This evidence, together with the fact that none of his second spermatocyte figures show any indication of the presence of a tetrad [His chromosome 'b' (p. 488 *C*, see my text fig. 3, 4) looks like any ordinary dyad and not a tetrad], has convinced me that his 'hexad' is a J-shaped tetrad. This would simplify matters, as on Voïnov's interpretation there are two sex-determining mechanisms present:—an accessory which forms part of the 'hexad' and in addition an unequal pair which he believes to be an XY. This leads him to remark (p. 496): "C'est un cas très rare, comme celui de *Banasa calva* parmi les Hémiptères, où de meme, grâce á la coexistence de deux chromosomes sexuels, naissent quatre catégories de spermatides, distinctes matériellement."

In 1905 Wilson reported the presence of an accessory and an idiochromosome pair in *Banasa calva*, but in 1907 he published

a note (which apparently Voïnov has not seen) in which he identifies as a supernumerary the element that he had formerly reported as the accessory.

Payne found what he believes to be a sex group in *Gryllotalpa borealis*. That is, an accessory and an unequal pair, the larger member of which always moves to the same pole as the accessory.

McClung has recently been able, through a knowledge of the J-shaped tetrads, to solve the puzzle of the multiple in *Mermeria* where he reported ('05), p. 316 that, "Entire tetrads pass into the second spermatocytes." It is now evident that the accessory is associated with a J-shaped tetrad which divides in the usual manner. Since apparently the accessory always attaches to the telomitic dyad, there would seem to be formed a sex-linked association comparable in end results with Payne's interpretation of conditions in *Gryllotalpa borealis*, the difference being that a physical union is lacking in the latter case. Voïnov's figures of his XY pair (plate 24, fig. 29, see my text fig. 3, 6) strongly suggest the association of a third element with a normal tetrad; making this a true hexad multiple. A study of Wilson's figures of idiochromosome pairs suggests that they also might be hexad multiples; i.e., that the X is the accessory plus one dyad of a tetrad of which the homologue is the Y. Since we know tetrads composed of unequal homologues exist, there is no reason apparent why the accessory might not be associated with either the larger or smaller member. This might account for instances in *Drosophila*, where the Y is larger than the X. This explanation of the known facts would seem simpler than that of Wilson ('11), who attempted to derive all from a primitive X-Y pair.

It may be well to note the bearing of the evidence as to the time of segregation brought out by this work on Bateson's 'Reduplication hypothesis.' Bateson ('15) in a study of the sweet pea found coupling of two pairs of factors which he represents by the formulae A a and B b, giving the heterozygote, A a B b. The zygotic ratios obtained in F₂ showed that certain combinations between these factors occur more frequently than others. This led Bateson to postulate the theory that

the original zygotic cell divided into two similar cells, A a B b but that the second cleavage plane passed differently through these two cells so that one gave daughter cells A B and a b, the other A b and a B. Some of these four cells divided oftener than the rest so that more gametes of certain kinds were produced. As to the time of these segregation divisions he says (p. 299):

Moreover the excess of gametes of parental composition characterizing the coupling- and repulsion-series must certainly mean that the position of the planes of division by which the four quadrants are constituted is determined with regard to the gametes taking part in fertilization—though the relative positions of the constituents of the cells may perhaps be maintained throughout the history of the tissues, it is easier to suppose that the original planes of embryonic division are determined according to those positions than that their influence can operate after complex somatic differentiation has been brought about.

Morgan ('15) has shown that the series of facts dealt with by Bateson is the same as those treated by Morgan and his students under the term linkage and that they are open to the same explanation.

It is evident that if such a somatic segregation as Bateson advocates occurred in *Trimerotropis* or *Circotettix*, we would have different types both of spermatogonia and of first spermatocytes in the individual, i.e. the metaphases would show varying numbers of atelomitic chromosomes, instead of being constant as I have found them to be.

2. *Heteromorphism, supernumeraries and reduction in the number of chromosomes*

The more I have studied this unusual group of grasshoppers the deeper has grown the impression that all of its peculiarities are closely interrelated. As was pointed out elsewhere (p. 465) there is some ground for associating the formation of vesicles with a shifting of the point of fiber attachment and also with a weakening of the chromosome at that point. In one animal, division constantly occurred at the point in question. It is

conceivable that this weakness might result merely in a constriction such as marks certain homologues. Figure 38 *a* (plate 11), where one of the larger tetrads in a member of form B is dividing at such a point, favors this interpretation. This is the only instance of this sort found in animal number 38.

So far, in all of our work, we have never found one of the larger tetrads with unequal homologues. On the other hand such peculiarities occur rather frequently among the smaller ones. This may indicate that the smaller chromosomes are less important. That they may be in part dispensed with was shown by Wenrich ('16) who finds that his tetrad 'C' lacks both terminal granules in certain individuals; and it seems possible that chromosome number 2 has been entirely eliminated from *Circotettix*. The homologues of the larger tetrads may be so important that a zygote lacking in even a part of one cannot develop.

The further suggestion presents itself that irregularities in mitoses such as have just been described, may be responsible for the occurrence of supernumerary chromosomes. Gametes containing a complete dyad plus a portion of its homologue would probably be viable and one may readily imagine that the extra portion might become detached in a succeeding division and might then form a supernumerary. Such an element would probably contain merely a reduplication of factor loci already present in the two dyads from whose homologue it was derived and might therefore have no influence in heredity.³ On the other

³ Dr. P. W. Whiting has suggested that it may be of interest to geneticists to point out that chromosomes such as numbers 7 and 8 (plate 1), which are indistinguishable morphologically, behave as duplicates, corresponding to Shull's theory of duplicate genes, and not as tetraploid homologues, as Muller has suggested for Gregory's tetraploid *Primulas*, e.g. If $A A'$ equals the atelomitic dyads and $a a'$ their telomitic homologues on Shull's theory A would always segregate from a , and A' from a' , or telomitic from atelomitic, giving a 1-2-1 gametic ratio. On the other hand, if they were tetraploid homologues in Muller's sense, A might segregate from A' and a from a' just as frequently, giving a 1-4-1 gametic ratio. In other words we would sometimes find the atelomitic dyad of chromosome number 7 mated with the corresponding one of number 8 and the same for the rod-shaped homologues. Since this condition does not occur, we must conclude that their morphological resemblance does not involve their behaving as tetraploids.

hand a supernumerary might contain the locus for multiple allelomorphs, in which case it might have an influence. The varying sizes found are perhaps due to two causes: (1) origin from different tetrads, (2) degeneration. More than one-fourth of the population of form 'B' contain supernumeraries, and, though one would expect that they would automatically pile up, no individual has been found with more than two. It therefore seems evident that there must be some method of elimination.

Wilson ('09) believes the supernumerary chromosomes in the Hemiptera are additional small idiochromosomes, and states that they are about the same size, of like behavior and show (Wilson, '07) some degree of coupling with the small idiochromosome. The supernumeraries found in the course of my work could not have had such an origin, since the sex-determining mechanism in the grasshopper is the accessory and not an X-Y pair. They are constant in size in a given individual, but range in different individuals from the size of the accessory (plate 9, 62) to less than a fourth of that size. In behavior during the growth period they simulate the accessory. If Wilson's hypothesis (that they are derived from the sex chromosome) were correct, my individual number 62, which appeared to be an entirely normal male, should have been a female; for the essential difference between the sexes is the possession of two accessories by the female, and in this particular case the supernumerary, in the first spermatocyte metaphases simulates the accessory so closely both in size and appearance, that it is sometimes difficult to decide which is which. It seems probable that it was a large supernumerary of this type that Davis ('08) confused with the accessory when he described two 'monosomes' (accessories) as being present in one specimen of *Arphia tenebrosa* (p. 102).

3. Taxonomic variability and gametic composition of the group

My own slight knowledge of taxonomy, together with a recognition of similarity of habits and environment acquired through field work, enabled me to group these related forms as they were collected. The specimens were later turned over to

Mr. J. A. G. Rehn of the Academy of Natural Science for identification. My study of the germ cells was completed, the plates made and the curves plotted before I knew the result of Mr. Rehn's study. Similarly, Mr. Rehn classified the animals without knowledge of my results, or even of the localities from which the various specimens were taken. It is but fair to say that, owing to lack of time, all that Mr. Rehn attempted to do was to place the animals in the classification already established, although he hopes soon to undertake a revision of the group.

One of the most striking differences shown by the study of the germ cells is the apparent reduction in number of chromosomes in the form which is unquestionably *Circotettix*. There is no conflict here between taxonomic and cytological evidence. Two species come in this division, *lobatus* and *rabula*. Unfortunately, I have only a single member of the latter species and the present work has shown that any attempt to establish specific differences on one or two individuals is futile for this group.

The remaining eighty-three members of this group may be placed in two subdivisions, according to the number of atelomitic chromosomes in the duplex series as is shown in text figure 1 (page 463). The mode for one of these subdivisions is twelve, for the other seven. There is an overlapping of the two groups involving twenty individuals, ten from each form. But since the extremes of neither form reach to the mean of the other they might constitute two distinct species. However, since these groups are from widely separated localities, the differences may well be due to isolation. If we consider what the expected progeny of parents like number 60, form B, with its five J-shaped tetrads would be, it is evident that some of the offspring would be placed in form A according to this scheme.

It is interesting to note that all members of my form A were classified by Mr. Rehn as the *Trimerotropis fallax* of recent literature. On the other hand he placed three of the fifty-one members of my form B in this same species. The remaining forty-eight were identified as *Circotettix suffusus* as that species is at present recognized. He states in a note that this is a "Divergent *Circotettix* tending strongly towards *Trimerotropis*."

From my cytological studies, I do not hesitate to say that form B is a Trimerotropis, and furthermore, it is a question if *Circotettix suffusus* and *Trimerotropis fallax*, as they are now recognized, do not even constitute a single species.

4. Correlation of chromosomal behavior and Mendelian principles

The evidence pointing to the chromosomes as the bearers of the heredity determiners has been summarized so often recently that I shall not repeat the process. One of the chief difficulties that cytological research has met with has been the impossibility of distinguishing between the chromosomes derived from the two parents. While it has been clear that homologous chromosomes segregate into different gametes, it has been impossible to say, except in the cases of the tetrads composed of unequal dyads recently reported (Carothers '13, Wenrich '14, Robertson '15), that all of the chromosomes brought in by the egg do not pass into the female-producing spermatozoon, as was suggested by Payne ('09) as a result of his study of *Grylotalpa borealis*.

Attempts have been made to determine the behavior of the paternal and maternal chromosomes in the maturation divisions and also to correlate given chromosomes with given somatic characters. Heretofore, the most promising line of attack on such problems has been through hybridization of forms with morphologically different chromosomes. The most noted of these experiments is that of Moenkhaus ('05). He crossed *Fundulus heteroclitus* and *Menidia notata*. The former has long, straight chromosomes, while those of the latter are short and curved. In the first few cleavages of the hybrid zygote, the chromosomes derived from one parent remained separate from those derived from the other. In later divisions the chromosomes became mixed on the spindle. But the important fact was demonstrated that the chromosomes derived from each parent maintained their morphological identity. The embryos did not develop beyond the closing of the blastopore.

Perhaps the most significant recent work along the line of hybridization is that of Federly ('13) who has combined breeding

and cytology in his study of the moth, *Pygaera*. He crossed *P. anachoreta* and *P. curtula*, and found by a study of the spermatogenesis of the hybrid, that pairing occurs between only two chromosomes; thus in the first spermatocyte of the parent forms there are twenty-nine or thirty chromosomes, while in those of the hybrid there are forty-eight of about one-half the size of the parental forms. When a back cross was made with one of the parent species, normal pairing took place between about one-half of the chromosomes of the hybrid and those of the parent species to which the cross was made, giving about thirty large paired chromosomes among a corresponding number of small unpaired ones. As one would expect from this cytological knowledge, both the primary hybrids and those resulting from the back cross were intermediate in all characters except one or two which showed normal dominance and segregation. The difficulty in working with such a form lies in the large number of chromosomes and in the lack of any means of distinguishing between them.

The work of Morgan and his students on *Drosophila ampelophila* is too well known to need much discussion. Here there is the advantage of a small number of chromosomes which differ in size and shape. According to Metz ('14) there are four pairs as follows: a pair of microchromosomes, a pair of sex chromosomes, equal in the female (XX), unequal in the male (XY), and two large V-shaped pairs.

Breeding tests with numerous mutants have shown one large group of genes, over thirty in number, to be sex-linked, hence borne, presumably, in the X chromosome. Two other great groups of over twenty members each, segregate independently and are assumed to correspond to the two large euchromosomes. Two characters so far—bent wings reported by Muller ('14) and eyeless, by Hoge ('15)—have been found whose genes segregate independently of the other three groups. Should mutations occur with equal freedom at any locus in the chromatin it would be expected, as pointed out by Muller, that the small microchromosomes would show fewer mutations than the larger chromosomes, and since the genes for bent wings and eyeless must lie

in one of the three pairs of euchromosomes, the presumption is that these two genes, rather than either of the two large groups of non-sex linked genes, lie in the microchromosomes. Should such prove to be the case, these would be the first instances of definite somatic characters being associated with a definite, recognizable euchromosome.

Dr. McClung, in two papers ('05 and '08), pointed out the general possibilities of cytological work and stated clearly his ultimate aim (p. 326, '05), "To determine the relation between individual chromosomes and characters in the body." For such a purpose the advantages of a species like *Trimerotropis fallax* or *Circotettix lobatus* is obvious. Taxonomically, there is striking individual variation, and correlated with this, apparently, are definite morphological variations, already described, of the homologous maternal and paternal chromosomes. Here, then, we have in a single species the means of distinguishing between certain homologous chromosomes. There is the further advantage of freedom from the sterility and lack of viability (if one may judge by the plentifulness of the animals) usually associated with hybrids. Of course it follows, that any ratios obtained through genetic work on these forms should be accurate, whereas those based on hybrids where there is a high degree of mortality, cannot be reliable.

So far no attempt has been made to breed these species, all of which, unfortunately for a worker located in the east, are Rocky Mountain forms. Another species of *Circotettix* occurs in mountainous regions of the east and, should it be as favorable cytological material as the western forms, I hope to do breeding work with it.

It might be said in passing that, according to my experience with several local species, grasshoppers are proving very favorable subjects for breeding. They stand captivity well and can accommodate themselves to various food and temperature conditions. They show strongly marked characters, breed freely, and it is possible to obtain three or four generations a year, at least of some species, by keeping them constantly at about 39°C. Special laboratory facilities, while convenient, are by no means essential since the eggs of most species normally live

through the winter out of doors and even the adults endure great extremes of temperature. The prime requisites for this work, aside from the 'hoppers' themselves, are fine wire cages and patience.

In any case, a study of a collection of individuals living in their normal environment was desirable, for, had such conditions first been found in captive stock, one would surely have suspected that these unusual conditions were due to the artificial environment.

This work has been sufficient, I believe, to show the random segregation of the homologues of all but four (nos. 2, 10, 11 and 12) of the first spermatocyte tetrads, and that the recombinations found in the group correspond to what would be expected according to the law of chance.

V. CONCLUSION

This material clearly presents the following facts:

1. Constancy of the chromosome complex for the individual.
2. The occurrence of heteromorphic tetrads in the first spermatocyte.
3. The segregation of these heteromorphic homologues in the first maturation division according to the law of chance.
4. The formation of the expected classes of second spermatocytes.
5. Normal transformation stages for the spermatids.
6. The occurrence in a group of individuals of practically all possible combinations of the heteromorphic chromosomes.

These facts taken together may be accounted for by:

1. The ordinary process of free, chance fertilization acting in a species in which the present forms of the chromosomes which originated through some past reorganization are stable, or:
2. A reorganization of the complex, involving a change of fiber attachment for each individual before the setting off of the germ cells.

Both of these possibilities may be tested by breeding:—In the first case parents of known chromosomal constitution would give progeny with a fixed range of variation—limited by the

number of possible combinations of the morphologically different gametes of the parents.

In the second case, any variation found in the group might occur in the offspring of a single pair.

The first explanation is a simple, logical one which meets all known facts; hence, in accordance with scientific custom, we are justified in accepting it for the present.

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EXPLANATION OF PLATES

The drawings were made with the aid of the camera lucida at a magnification of 2400 diameters and have been reduced one-third in reproduction.

Plates 1 to 10 show side views of first spermatocyte metaphases, arranged as nearly as possible according to size. Each horizontal row represents one cell, the vertical rows, corresponding chromosomes in different cells. They are so arranged that the accessory, number 4, is always passing to the upper pole.

The individuals from which drawings were made were numbered consecutively from one to seventy-five. The same number always applies to the same specimen; successive figures from any individual are indicated by letters.

When supernumerary chromosomes are present they are placed at the left of chromosome number 12 to indicate that they are out of the normal series and especially because they would occupy no definite position in relation to size.

Plate 14 is composed of photomicrographs made at a magnification of 1140 diameters and reproduced without reduction.

PLATE 1

EXPLANATION OF FIGURES

Lateral views of first spermatocyte complexes from a single individual (no. 1), showing mode of segregation of three heteromorphic tetrads (chromosomes no. 1, 7, and 8.

b to *e* Alternate distribution of atelomitic dyads of chromosomes number 7 and number 8.

f and *g* Concurrent distribution of same homologues segregating opposite the accessory. In *f* the atelomitic dyad of chromosome number one is accompanying the similar dyads of chromosomes number 7 and 8, while in *g* it is passing to the opposite pole.

h and *i* Same as *f* and *g* except that atelomitic dyads of numbers 7 and 8 are passing to the same pole as the accessory.

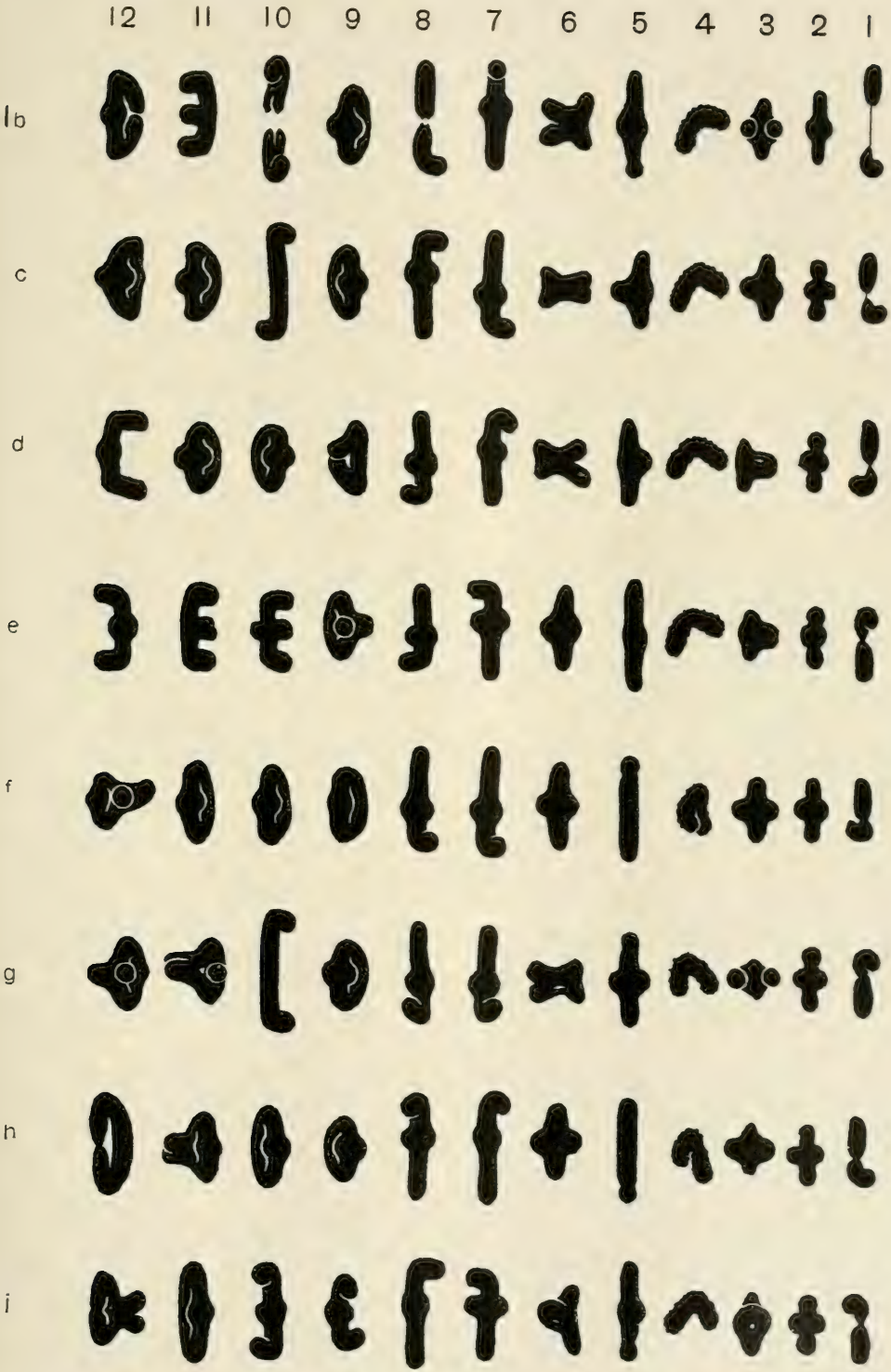


PLATE 2

EXPLANATION OF FIGURES

Side view of first spermatocytes from eight individuals of form 'A,' showing variation of complex from individual to individual.

6 An animal with eight chromosomes of the *Stenobothrus* type.

7 A different individual with only four such chromosomes. The other four being replaced by J-shaped tetrads.



PLATE 3

EXPLANATION OF FIGURES

Side views of eight first spermatocytes of form 'A' showing variation within the group.

10 Two supernumerary chromosomes, one large the other small.



PLATE 4

EXPLANATION OF FIGURES

Side views of first spermatocyte complexes from eight individuals of form "A." Showing variation within the group.

22 An animal showing heteromorphic condition of chromosomes number 11 and number 9.

23 Homomorphic short armed form of chromosome number 9.

24 Homomorphic long armed form of chromosome number 9 and heteromorphic form of chromosome number 11.

25 Homomorphic telomitic form of chromosome number 9.

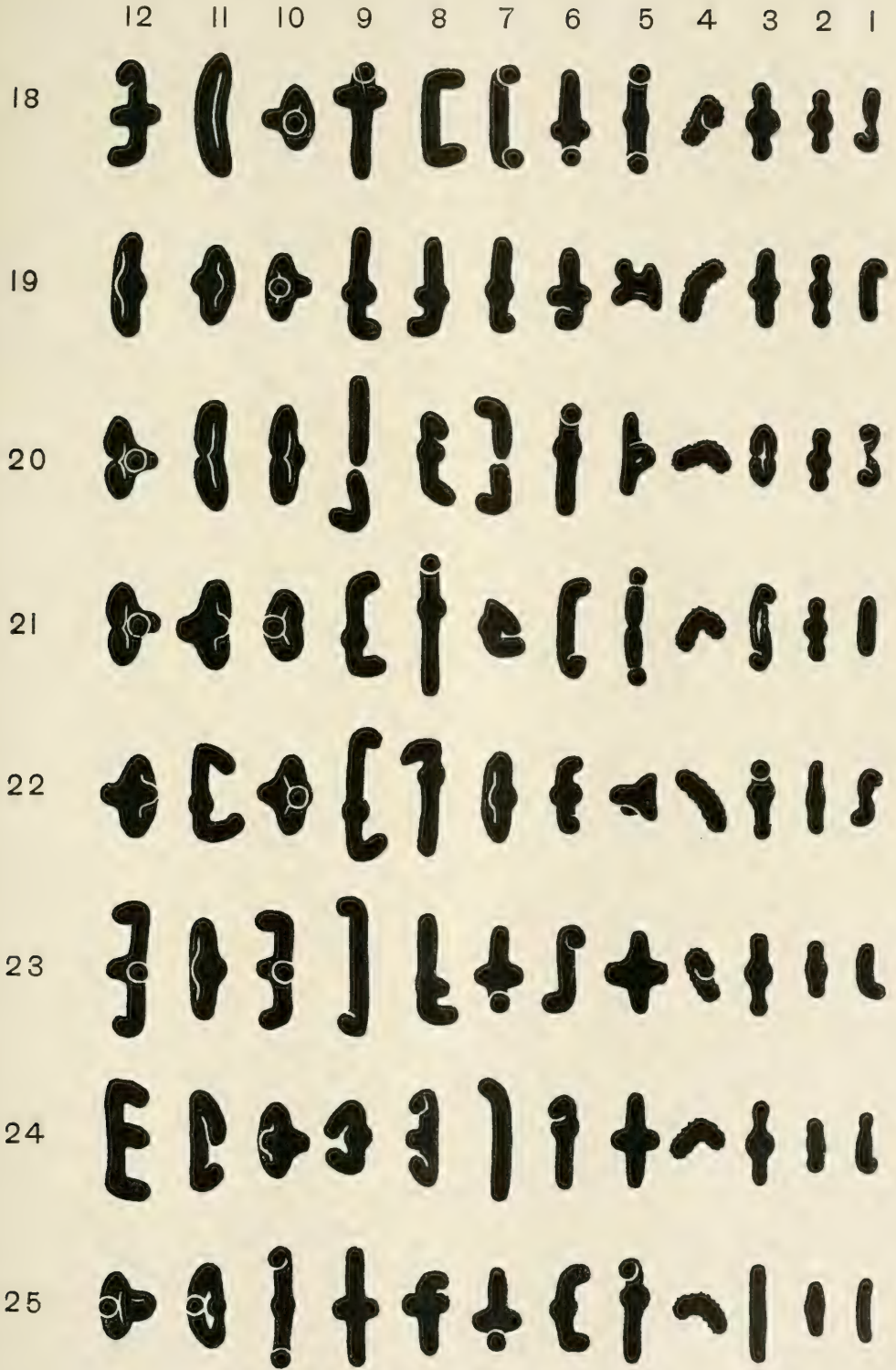


PLATE 5

EXPLANATION OF FIGURES

Side views of seven first spermatocyte metaphases, form 'A,' showing variation within the group.

31 An individual with all of the chromosomes except numbers 1 and 2 either atelomitic or J-shaped.

32 An early anaphase with fifteen atelomitic dyads.

32a' Spermatogonial metaphase with chromosomes arranged in pairs showing fifteen atelomitic chromosomes. From same animal as 32. Plate 11, figure 32a, is the same complex as drawn under the camera lucida.



PLATE 6

EXPLANATION OF FIGURES

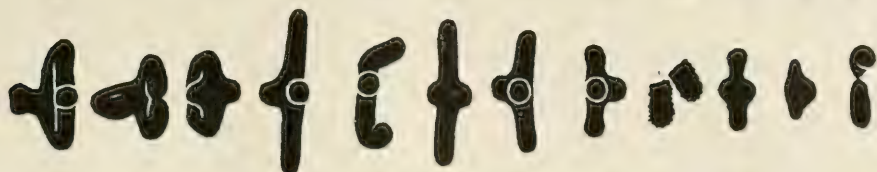
Side views of eight first spermatocyte metaphases, form 'B.' Note the difference in general appearance between plates 2 to 5 and 6 to 9, due to preponderance of atelomitic and J-shaped tetrads in former.

33 Chromosome number 4 (accessory) in two sections.

38 Chromosome number 10 broken.

12 11 10 9 8 7 6 5 4 3 2 1

33



34



35



36



37



38



39



40



PLATE 7

EXPLANATION OF FIGURES

Side views of eight first spermatocyte metaphases, form 'B.'

41 Chromosome number 4 (accessory) broken.

43 Two supernumeraries, one very large.

45 Accessory (no. 4) with numerous fiber attachments.



PLATE 8

EXPLANATION OF FIGURES

Side views of eight first spermatocyte metaphases, form 'B.'

36 Two supernumeraries.

12 11 10 9 8 7 6 5 4 3 2 1

49



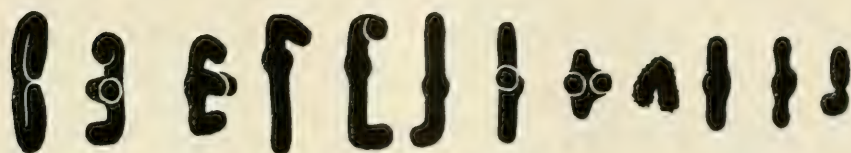
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51



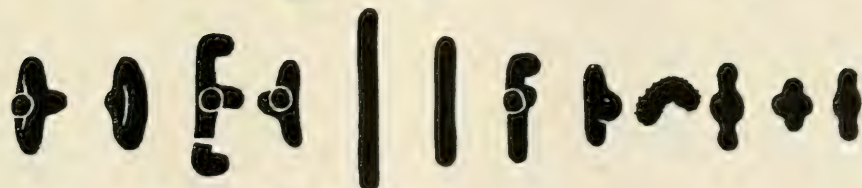
52



53



54



55



56



PLATE 9

EXPLANATION OF FIGURES

Side views of seven first spermatocyte metaphases, form 'B.'

62 This individual has the least number of atelomitic chromosomes found. Seven in the spermatogonia, resulting in three rings of the *Stenobothrus* type and the usual atelomitic accessory in the first spermatocyte. Note the very large supernumerary.

63 An early anaphase—chromosome number 4 cut.

63a' Spermatogonial metaphase with chromosomes arranged in pairs. From same animal as 63. Note the very close resemblance to the first spermatocyte anaphase especially. chromosome number 8, one homologue of which is constricted in each, and chromosome number 7, one homologue of which is atelomitic in both cases.

For the four classes of second spermatocytes formed by this individual, see plate 13, 63b to e.



PLATE 10

EXPLANATION OF FIGURES

Side views of eight first spermatocyte metaphases of *Circotettix lobatus*.
Note that there are only eleven chromosomes, number 2 being absent.
None of these individuals have less than four rings of the *Stenobothrus* type.



PLATE 11

EXPLANATION OF FIGURES

Spermatogonial and somatic metaphases.

1i From the same animal as plate 1, twelve atelomitic chromosomes.

22a Seventeen atelomitic chromosomes. For first spermatocyte, see plate 4, 22.

32a Fifteen atelomitic dyads. For rearrangement and comparison with first spermatocyte, see plate 5, 32 and 32a'.

33a Ten atelomitic chromosomes.

38a A tetrad dividing unequally.

62a Seven atelomitic chromosomes, the minimum number.

62b Another complex from above individual.

63a Eight atelomitic chromosomes. For rearrangement and comparison with first spermatocyte, see plate 9, 63 and 63a'.

70a *Circotettix lobatus*. Entire complex, twenty-one chromosomes.

70d Prophase tetrad showing vesicles on one homologue.

70e Unequal division of same tetrad in first spermatocyte metaphase.

75 *Circotettix lobatus*. Entire complex, twenty-one chromosomes.

72 Somatic complex from a female of form 'B,' ten atelomitic chromosomes.

73 From another female of same group, eight atelomitic chromosomes.

74 From a female of form 'A,' fourteen atelomitic chromosomes.

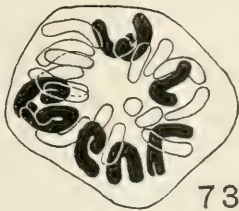
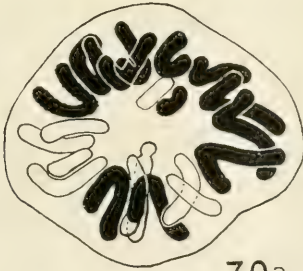
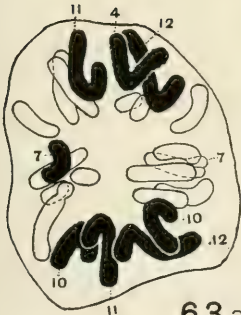
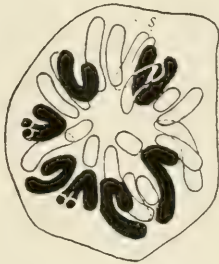
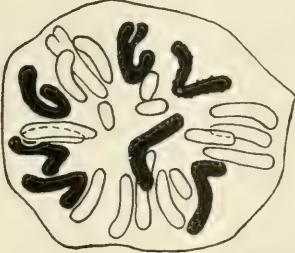
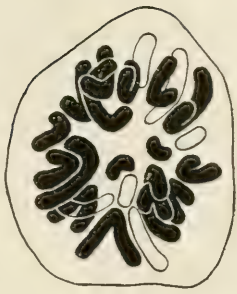
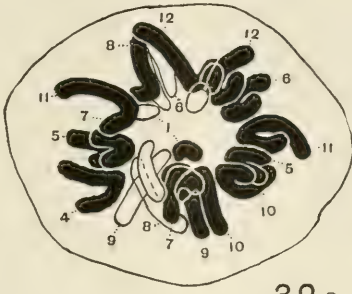
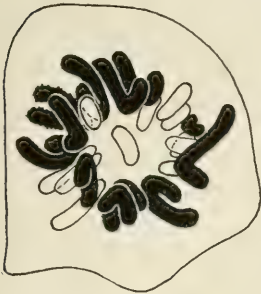


PLATE 12

EXPLANATION OF FIGURES

Second spermatocyte metaphases from animal number 1 showing twelve morphologically different types, as follows:

Eleven chromosomes

1. *j*, four V's plus seven rods.
- k*, four V's plus six rods plus V of tetrad number 7 or 8.
- l*, four V's plus six rods plus V of tetrad number 1.
- m*, four V's plus five rods plus V's of numbers 1 and 7 or 8.
- n*, four V's plus five rods plus V's of numbers 7 and 8.
- o*, four V's plus four rods plus V's of numbers 7 and 8.

Twelve chromosomes

- p*, five V's plus seven rods.
- q*, five V's plus six rods plus V of tetrad number 7 or 8.
- r*, five V's plus six rods plus V of tetrad number 1.
- s*, five V's plus five rods plus V's of numbers 1 and 7 or 8.
- t*, five V's plus five rods plus V's of numbers 7 and 8.
- u*, five V's plus four rods plus V's of numbers 1, 7 and 8.

For first spermatocyte metaphases from this animal, see plate 1, and for spermatogonia plate 11, 1 *i*.

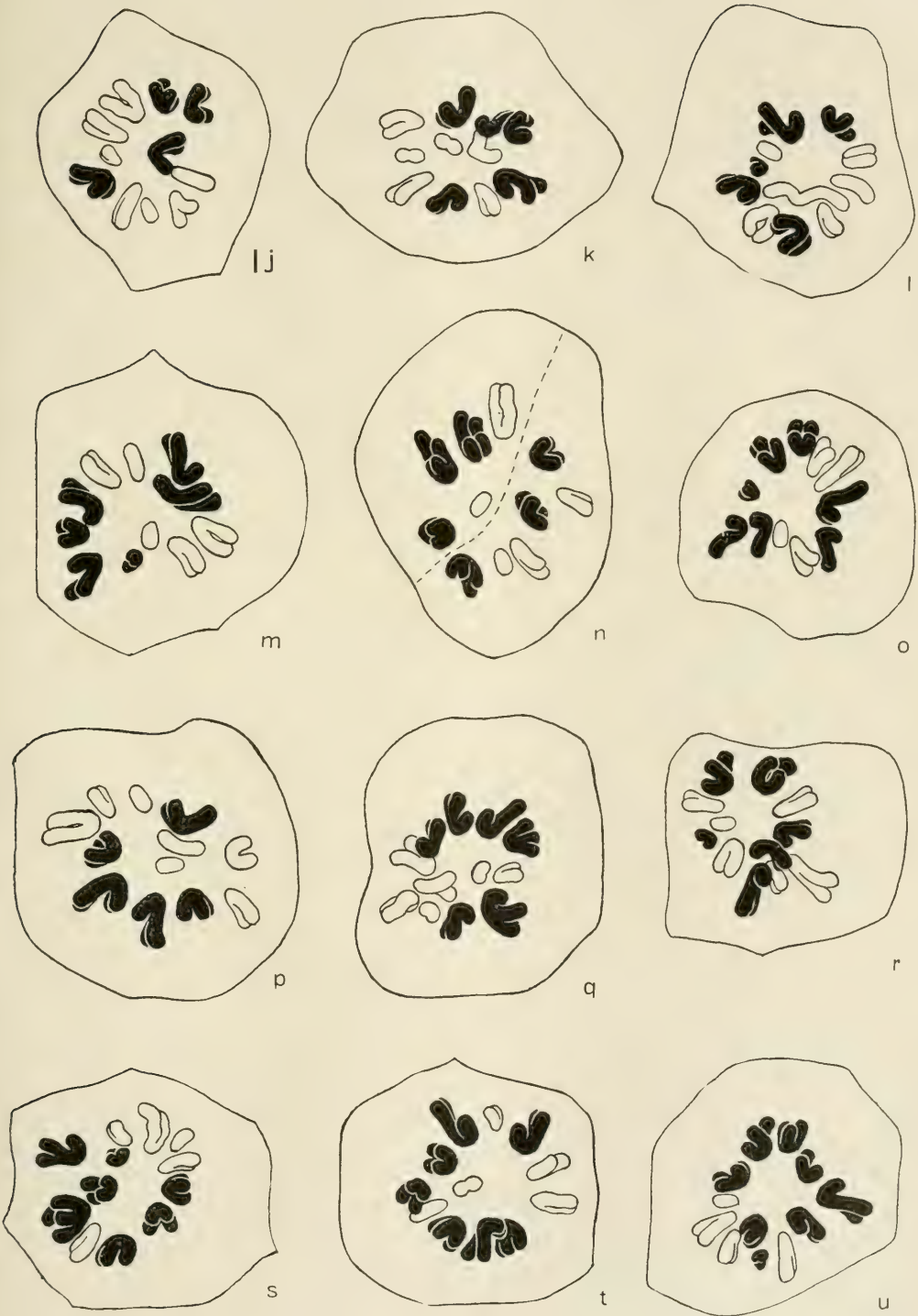


PLATE 13

EXPLANATION OF FIGURES

Second spermatocyte metaphases.

62*b* to *c* From the individual with the minimum number of atelomitic chromosomes, seven in spermatogonia (Plate 11, 62*a*). For first spermatocyte see Plate 9, 62. There are four types owing to the presence of the supernumerary as follows:

b, eleven chromosomes.

c, eleven chromosomes plus supernumerary.

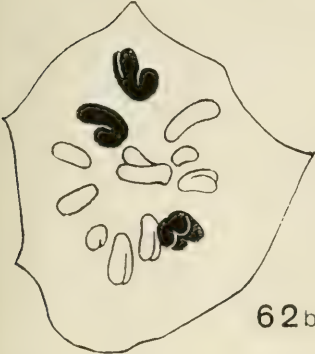
d, twelve chromosomes.

e, twelve chromosomes plus supernumerary.

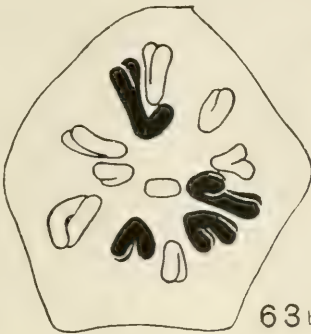
63*b* to *e* The four classes of second spermatocytes formed by individual 63.

69 *Circotettix lobatus*, eleven chromosomes.

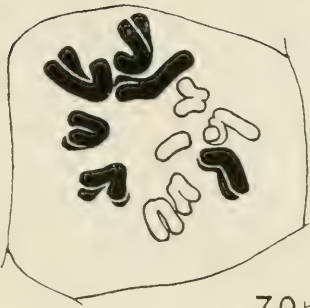
69*b* Same, ten chromosomes.



62b



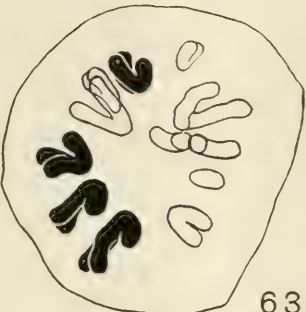
63b



70b



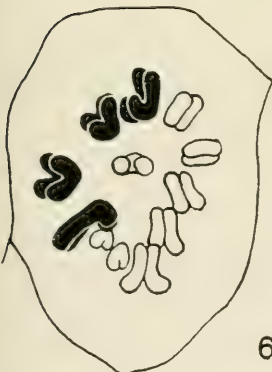
62c



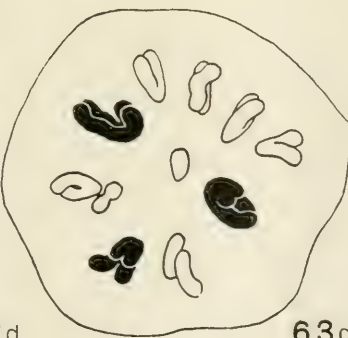
63c



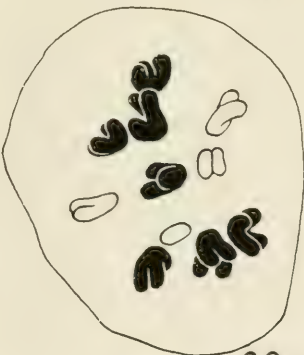
70c



62d



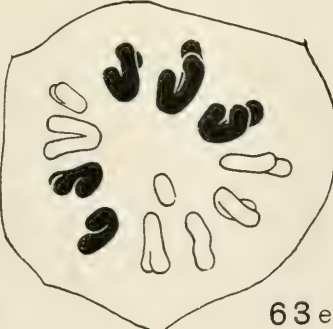
63d



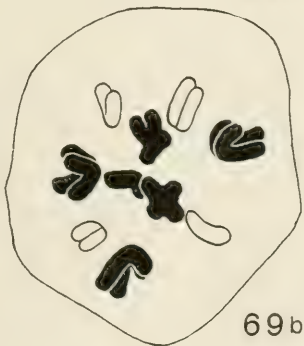
69a



62e



63e



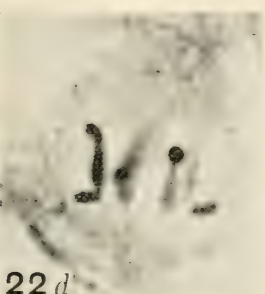
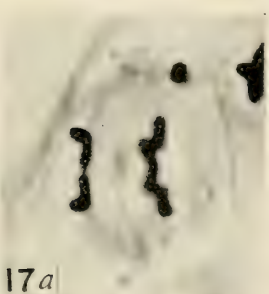
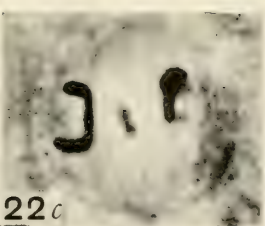
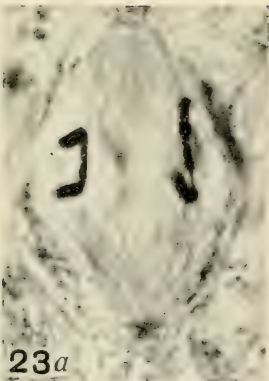
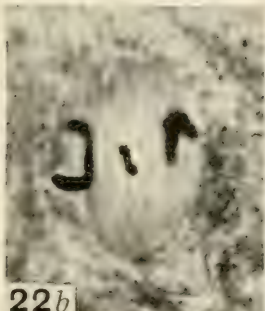
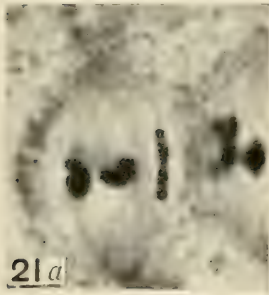
69b

PLATE 14

EXPLANATION OF FIGURES

Photomicrographs

- 21a Homomorphic constricted type of chromosome number 3.
- 62f Heteromorphic form of same chromosome from another individual—one homologue constricted the other smooth.
- 13a Homomorphic smooth type of same chromosome from a third animal.
- 1v The concurrent passage of the atelomitic homologues of chromosomes number 7 and 8 to the opposite pole from the accessory in specimen number 1.
- 1w The same except that the atelomitic dyads are passing to the same pole as the accessory.
- 1x The three J-shaped tetrads of animal number one with alternate distribution of atelomitic dyads of chromosomes number 7 and 8.
- 22b Homomorphic, atelomitic form of chromosome number 1.
- 22c Heteromorphic condition of chromosome number 11.
- 23a Homomorphic long-armed form of chromosome number 11.
- 10a Homomorphic short-armed form of chromosome number 11.
- 22d Heteromorphic condition of chromosome number 9.
- 17a Homomorphic long-armed form of chromosome number 9.
- 10b Homomorphic short-armed form of chromosome number 9.



THE CHROMOSOMES OF THE COMMON HOUSE MOSQUITO, *CULEX PIPIENS* L.

P. W. WHITING

Zoological Laboratory of the University of Pennsylvania¹

SEVEN TEXT FIGURES AND SEVEN PLATES

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¹ Contribution from the Zoological Laboratory of the University of Pennsylvania.

1. INTRODUCTION

On account of the contradictory statements published in regard to the cytological conditions of the common house mosquito, *Culex pipiens* L., it was thought worth while to investigate the matter thoroughly in order to find out the cause of disagreement and to discover the true conditions. This has seemed of especial interest in view of the extensive genetic work that is being done on one of the higher Diptera, *Drosophila ampelophila* Loew.

While certain other orders of insects have been well investigated cytologically, the published work on the Diptera is very meager. It consists of two papers on mosquitoes by Stevens ('09 and '11), one by the same author on nine species of *Myodaria* ('08), a paper on *Culex pipiens* by Lomen ('14), another by Taylor ('14), and a paper on twelve species of *Drosophila* by Metz ('14). Besides these there are two short reviews, one by Metz ('16), and one by Dehorne ('14), and a genetic paper by Bridges ('16) dealing with non-disjunction of the sex chromosomes in gametogenesis of *Drosophila ampelophila* and figuring and describing the diploid complexes.

It was thought best to make a thorough study of the spermatogenesis of *Culex pipiens* and a comparative study of conditions in the ovary and somatic tissue. By reason of improved technique and the encouragement and criticism of Dr. C. E. McClung, Dr. D. H. Wenrich, and Miss E. Eleanor Carothers, I have been enabled to make considerably more out of the material than was at first expected.

A discussion of technique, together with a criticism of the literature, will be reserved until after the descriptive part of the paper. It is believed that the contradictory results obtained by the various investigators have been largely due to differences of technique and it is therefore desirable to discuss these matters after presenting my own results. It may merely be stated here that Flenning's stronger fluid has been used as a fixative except where otherwise mentioned. *Culex pipiens* may be bred in large numbers throughout the winter months and quantities of material obtained at all times for study.

II. OBSERVATIONS

A. SPERMATOGENESIS

a. Introductory

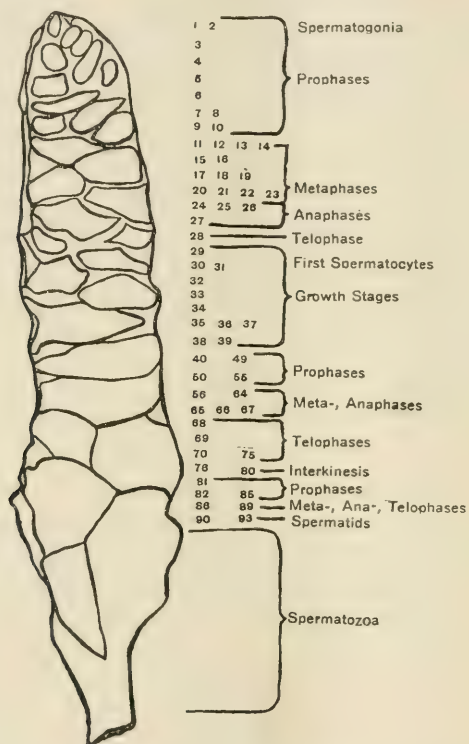
The testes of *Culex pipiens* are two ellipsoidal, colorless, highly refractive bodies lying laterally in the sixth abdominal segment. Each consists of a single follicle, divided by partitions of connective tissue into a number of cysts. The spermatogonia are formed in the cephalic end and these are followed by the successive stages of spermatogenesis as shown in text figure 1, which is from a longitudinal section of a pupal testis at a magnification of 1800 diameters. The numbers to the right of the figure refer to the successive stages as illustrated by figures 1 to 93. Usually not all stages will be found in a single testis, but I have occasionally found an individual with divisions of spermatogonia, first and second spermatocytes, and with spermatids. The development of the germ cells is not completely correlated with the age of the insect, but considerable variation obtains.

b. Spermatogonia

The last spermatogonial division and the 'resting' nuclei preceding it are the earliest stages found in full grown larvae and pupae. Probably younger larvae would show a greater number of spermatogonial generations.

1. *Prophases*. The earliest stage obtained is illustrated by figures 1 and 2. The chromosomes are very thin and only partly visible. A large irregular, diffuse nucleolus is a striking characteristic of this stage. It often shows four darker areas (fig. 1). There may be more than one nucleolus (fig. 2), together equivalent, however, in size and staining qualities to the one. The nucleolar elements are clearly attached to the chromatic threads, which may sometimes be traced to the periphery of the nucleus. They show no tendency to be approximated to the side of the nucleus, but occupy various positions in the karyolymph.

Successively later stages, as judged by relative position of the cysts, are shown in figures 3, 4, and 5. The nucleolar elements are darker in these stages. They tend to appear separate at first (fig. 3), and to fuse later. The nucleolus then gradually becomes diffuse while the chromosomes thicken and stain more densely. Figure 6 represents an optical section of a nucleus at



Text Figure 1. Location of stages in the gonad

this stage. The separate chromosomes can not yet be made out, but a thick chromatic cord is attached to the diffuse nucleolus. Two terminal granules, each connected to the tip of the chromatic thread to which the nucleolus is attached, are of interest as they appear again in first spermatocyte prophase. They are here diffusely stained and suggest by their form the antennae of a butterfly.

Upon the disappearance of the nucleolus the separate chromosomes become apparent as three pairs of bent threads. The nuclear membrane now dissolves and the chromosomes appear as in figures 7, 8, and 9. Two of the pairs are very much longer than the third, but this difference is not always well seen in the figures on account of foreshortening due to twisting up or down. Figure 9 is a slightly later stage than the others and the chromosomes have shortened and thickened. Figure 10 is a very late prophase.

2. *Metaphase, stages a and b.* Figures 11, 12, 13, and 14, represent a very characteristic arrangement of the chromosomes in a flat plate with a somewhat radially symmetrical appearance. This stage may be called stage A of the metaphase. The chromosomes do not begin their division at the end of this stage but first assume a parallel arrangement, which may be called stage B. In stage A the chromosomes tend to be constricted in the middle and swollen at the ends. The homologues are usually approximated and their middles are nearer to each other than their ends. Since the chromosomes are in an approximately flat plate we may speak of a polar or an equatorial view of the same by analogy with the metaphase. Equatorial views are not favorable for drawing, but the chromosomes may be studied and counted by focusing.

In the figures of stage A a short line is drawn to represent the polar axis. The number at one end of the line denotes in degrees of the circumference of a circle the slope of the polar axis to the plane of the drawing. The direction of the polar axis has been estimated by focusing upon the metaphase plate.

The smaller pair of chromosomes is readily distinguishable from the larger pairs in metaphase and anaphase, as it is also in late prophase. There is considerable variation in the size of the chromosomes from different cells, probably depending upon nutritive conditions or previous rapidity of cell divisions.

There are certain points of interest to be noted here in connection with diffuse bodies and extra elements. In figure 9 there is represented an extra darkly stained element alongside one of

the big chromosomes in the lower right hand corner. Nothing of the sort can be made out in the cell shown in figure 10. In figure 11 is drawn a diffuse body resembling the nucleolus. Figure 12 also shows a diffuse body as well as a diffuse element attached to the outer member of one of the large pairs of chromosomes. In figure 13 are two diffuse bodies, and in 14 a diffuse end of the inner member of a large pair. This end is much more condensed than that shown in figure 12. In stage B of the metaphase and in anaphase these diffuse bodies and extra elements are not seen.

Figure 15 is a condition frequently observed, in which the chromosomes are passing from stage A to stage B. The members of the small pair are still considerably bent, those of one of the larger pairs appear to be perfectly straight. The radial symmetry of stage A is here lost and the six chromosomes are beginning to assume the parallel arrangement of stage B.

Figure 16 shows a metaphase in which the chromosomes have assumed an approximately parallel arrangement. In the description of this stage and the stages immediately following, it will be necessary to distinguish three axes—the polar axis and two equatorial axes. The polar axis connects the poles of the spindle and passes through the centre of the metaphase plate and at right angles to it. An equatorial axis cutting the chromosomes transversely may be called the transverse axis, while an equatorial axis lying parallel with the chromosomes and passing between the members of the middle pair may be called the parallel axis. In figures 16 to 28 the directions of the three axes are represented by lines, each with a number at one end, which indicates how many degrees of a circumference the end of the line should be raised above the plane of the paper to get the inclination of the axis. The polar axis is represented by a solid line, the transverse axis by a dotted line, and the parallel axis by a dot-and-dash line. Thus figure 16 is a polar view, with the transverse and the parallel axes in the plane of the paper. The three axes are of course always at right angles to each other. If we disregard differences of size and irregular bending of the chromosomes, we see that in stage B the meta-

phase plate approximates biradial symmetry, the transverse and parallel axes each dividing symmetrical halves.

Figure 17 is the same stage. The shortness of the chromosomes is due in part to sloping of the polar axis, in part to fixation. In figure 18 the shortness of the chromosomes is also due to shrinkage, illustrating the effect of imperfect technique. The figure shows that the small pair may occupy the centre of the metaphase plate. In figure 19, a transverse optical section of the chromosomes in the same stage, the members of each pair are closely approximated, apparently a matter of fixation. Figures 17, 18, and 19 are all from the same testis which showed imperfect fixation. The small pair in figure 19 is on the left, as may be judged by focusing, although the diameter is as great here as that of the others.

Figure 20 shows that the chromosomes begin to split in the middle. The plate is so bent that, although the transverse axis is in the plane of the paper, five of the chromosomes show the beginning of the split. Figure 21 is a similar stage. The chromosome at the right has been pulled out of position by sectioning and broken at the point of attachment of the spindle fibres; the daughter chromosomes are somewhat separated. Figure 22 is a side view of a chromosome from another plate at this same stage and figure 23 is a transverse optical section of a similar plate. The ends of the chromosomes seen at the left and right are visible on account of bending. Figure 24 is a side view of a chromosome in a little later stage of division showing part of another chromosome that was in the same optical section. Figure 25 is an oblique view of the chromosomes at a slightly later stage,

3. *Anaphase and telophase.* Figure 26 is an optical section of the ends of the chromosomes just separated in anaphase. Figure 27 shows a later anaphase with the chromosomes still displaying some of their parallel arrangement.

The spermatogonial chromosomes always seem to pull out very thin in anaphase and the members of the homologous pairs are often so close as to be inseparable. In figure 27 the members of the small pair have apparently fused completely.

Bending of the polar axis frequently takes place in anaphase, and in telophase the daughter nuclei may become considerably displaced relative to each other. Figure 28 represents a telophase in which considerable bending of the polar axis has taken place. The directions of the three main axes are therefore given separately for each newly-formed nucleus. The chromosomes still have their arrangement parallel to each other in each daughter nucleus. This is clearly seen at the right but not at the left, as the equatorial axes are sloping.

There is considerable tendency for the chromosomes to become diffuse in anaphase, and in telophase this has gone so far that, with normal staining by the iron-haematoxylin method, the nuclei appear quite devoid of any chromatic material. The slide from which figure 28 was taken was very darkly stained. The telophase nucleus gives rise to that of the first spermatocyte at the division of the cell body.

c. First spermatocytes and nucleolar elements

1. *Earlier prophases.* The character of the first spermatocyte nucleus shortly after the last spermatogonial telophase is shown in figure 29. The chromosomes are exceedingly tenuous and the nucleolus is very diffuse, with four darker areas. Except for its smaller size the nucleus resembles that of the spermatogonia as shown in figure 1. At a little later stage, as judged by position of cysts, the nucleolus has condensed into two black masses (figs. 30 and 31). Figures 32 *a*, 32 *b*, and 33 show later stages in which the chromosomes are more condensed, nuclear size considerably increased and nucleolus irregular and somewhat diffuse in parts. In figure 34 the nucleolus is very black, but has a light centre. At this stage the nucleolus is always very smooth in outline. Figure 35 is a characteristic condition of a later stage in which the dark area of the nucleolus is surrounded by a lighter granular investment. In figure 36 the diffuse part appears to have been separated from the darker part and forms a pair of terminal granules which are seen very frequently in later stages. In figure 37 the nucleolus has become diffuse, while the chromatic threads are thicker and darker.

The double thread terminating in the granules has become very much condensed near them. It is of interest to see that there are four threads connected with the nucleolus and that at least one of them may be continuous between the nucleolus and the terminal granules. Figure 38 represents a later stage in which the four single threads connected with the nucleolus have fused into two double threads.

In figure 39 the still diffuse chromosomes appear distinctly as three pairs, the smaller pair at the center of the figure and at a much lower focus than either of the others. The two larger pairs are at the sides with the members united at the ends. The pair at the right shows the terminal granules. The nucleolus is still very black and is at considerable distance from the pair at the right, but connected with the middle of the same by an achromatic fiber.

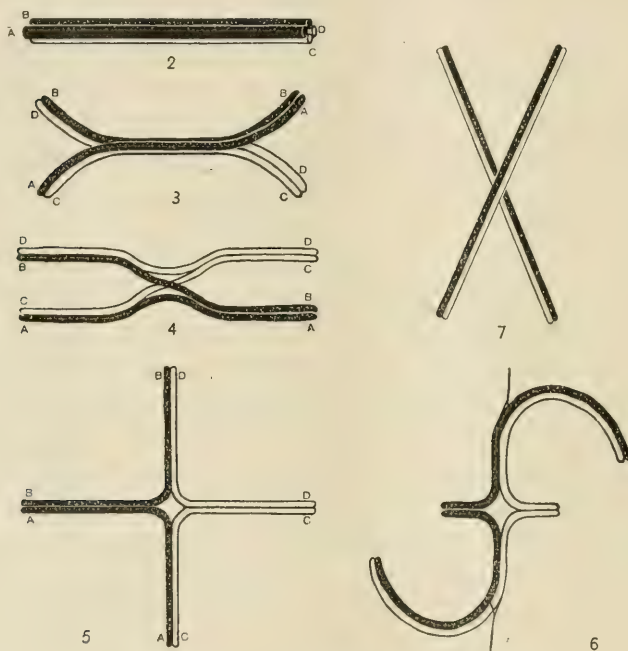
Figures 40 to 47 are from sections which have been very much overstained in order to bring out the more diffuse elements. In consequence the chromosomes are very black and the members of each of the three pairs appear more or less fused.

Figure 40 shows one of the larger pairs with the diffuse nucleolus attached to its middle and the terminal granules each appearing double. The more distal parts of the latter are very black. In figure 41 the terminal granules are rather large; the attachment of the nucleolus can not be made out. Figures 42 *a* and 42 *b* show a nucleus cut in sectioning. The terminal granules are here very small. The nucleolus, now appearing double, is connected with the large pair of chromosomes which bear the terminal granules. The two other pairs have been broken in the middle, the point of spindle-fiber attachment. Figure 43 shows the diffuse nucleolus and a terminal granule attached to the same large pair. The other terminal granule could not be seen but is probably hidden under the chromosomes. In figure 44 the nucleolus has become exceedingly diffuse and in figures 45, 46, and 47 is not visible. The terminal granules vary greatly in size, in 47 becoming hardly more than faint threads.

2. *Later prophases to early anaphases. Tetrads.* In order to make clear the conditions shown in the succeeding figures of

first spermatocytes, diagrams will be helpful. Text figure 2 represents a tetrad with its four elements, the chromatids. *A* and *B* represent the chromatids from one homologue and *C* and *D* the chromatids from the other.

The formation and existence of tetrads mark the post-spireme stage which ends, according to Wenrich ('16), "with the establishment of the tetrad-chromosomes upon the mitotic spindle of



Text Figures 2-7. Separation of the tetrad

the first spermatocyte division." Since, however, in *Culex pipiens*, each tetrad passes through late prophase stages, through the metaphase, and up to the anaphase independently of the others, the post-spireme stage must be considered separately for each tetrad.

In text figure 3 the tetrad has split at each end and the splits are at right angles to each other. To the right the homologues are separated by the primary split of Wenrich. To the left is shown the secondary split, separating the fused daughters of

homologues. If these splits be continued until they intercept each other, there will result a condition like text figure 4, in which one chromatid from each chromosome appears to cross over and unite with the other chromatid from the other chromosome. If we suppose the primary and the secondary splits to be widened so that the ends of the chromosomes are pushed apart until they all lie in the same plane, there will be formed a cross like text figure 5. The horizontal arms of the cross are homologues. The vertical arms are fused daughters of homologues. The spindle-fibers may be supposed to attach to the middles of the chromatids. If the chromosomes of text figure 5 separate in a horizontal direction the division will be reductional. If they separate vertically the division will be equational. Text figure 6 represents an equational division. Text figure 7 represents a simple crossing of two equationally divided chromosomes.

It now becomes evident that it is impossible to determine in *Culex pipiens* whether the first division is reductional or equational. In the first place the members of the pairs as they appear in late prophase of the first spermatocyte may represent homologues or they may represent fused daughters of homologues.

Figure 48 shows the three pairs at a somewhat later stage than figure 39. Where the members touch each other there may be a simple crossing like text figure 7, or a separation in different planes like text figure 4.

In figure 39 the ends of the larger pairs are united. It is impossible to tell whether the split which separates the middles is primary or secondary. In later stages appearances suggest very strongly alternations of splits at various points of the chromosomes. Where this occurs the chromatids may be said to be 'changing partners.' In figure 49, from another cell of the same cyst as figure 48, points *a*, *b*, and *c* are clearly simple crossings of the fibers; point *d* suggests very strongly a 'change of partners.'

Figure 50 is a later stage in which each of the three pairs shows a change of partners at one end. In pair *a* this has progressed well toward the middle. In this and the succeeding

figures, pair *b* is the small pair. Figure 51 shows that the chromatids of pair *a* are changing partners at both ends, thus forming a ring with a cross at each side. The difference in length of the transverse arms of the cross is a matter of perspective. Figure 52 shows the three pairs, each with a change of partners at one end. In *a* the split of the ends is in the plane of the paper and the lower point of union is bent down so that the chromatids uniting there appear shorter on account of perspective.

In figure 53 each of the three pairs has formed a ring, with a change of partners at each end. This is a typical form of prophase tetrad. The tetrads have been displaced for clearness in drawing. The direction and extent of displacement is represented by the direction and the length of the arrows.

In figure 54 each of the three pairs has formed a cross by a change of partners comparable to text figures 5 and 6. This is also a typical form of prophase tetrad. Tetrads of either the ring or the cross variety may thus be formed by any one of the three pairs.

In figure 55 *a* and *b* are tetrad crosses, while *c* has the members still separated. In figure 56 *a* and *b* are rings, the latter, however, incomplete at the lower focus. Tetrad *c* is a cross seen from side view.

The change from late prophase to metaphase and anaphase is passed through by each tetrad independently of the others. In figure 57 tetrads *b* and *c* are in early anaphase while *a* is still in prophase. The prophase tetrads are thicker and less clean-cut than those in metaphase. This is probably due to relative degree of condensation.

In figure 58 *b* and *c* are in late metaphase, showing clearly their fibre attachments. Tetrad *a* is a thick, irregular prophase cross without apparent fiber attachments. In figure 59 the small tetrad *b* is still in prophase, but the outlines are more clean-cut, showing a certain degree of condensation. In figure 60 the outlines of the cross-shaped tetrad *c* are smooth and a fiber attaches to one of its arms. Tetrad *a* has separated irregularly so that a part of the upper member is still attached to the member going to the lower pole.

In figure 61 all three tetrads are in metaphase condition. The small one, *b*, is shown at two foci.

Figure 62 is a late metaphase, and figure 63 a similar stage from a slide which was much decolorized. The separate chromatids are here plainly seen. Tetrad *c* strongly suggests text figure 6.

Figure 64 shows an early anaphase in which the small tetrad *b* has divided before the others.

The spindle fiber, wherever it can be distinguished, is always attached near the center of the dyads, consistent with the condition in the spermatogonia.

3. Later anaphases and telophases. When the dyads have separated in anaphase and are approximately at the poles, they split longitudinally, forming three pairs of V-shaped monads at each pole. In a side view of the anaphase spindle the chromosomes are often difficult to separate and inconvenient to draw, due to overlapping.

In figure 65, an oblique view of an anaphase, the dyads have already split into monads. The two poles of a slightly later stage are shown from polar view in figure 66. Figure 67 shows an optical section of a similar stage cutting through each arm of the monad V's. Such appearances are very characteristic of this stage.

Figure 68 is a slightly later stage when the chromosomes have reached the poles. The sister monads are drawn closely together, especially in their middles. At *a* is shown the upper pole; *b* is an optical cross section of the spindle fibers; and *c* shows the lower pole. The chromosomes at *c* are becoming somewhat vesicular at their ends. The sister monads tend to unite at this stage, beginning at the middle and progressing toward the ends.

Figure 69 shows five optical sections along the polar axis of a first spermatocyte telophase. The sister monads separated in the anaphase seem to have reunited in the telophase. In figure 69 *a* is at a higher focus than *b*. The numbers about the circumferences of *a* and *b* mean that the chromosomes opposite those in *a* are continuous with those numbered correspondingly in *b*.

The middles of the chromosomes are drawn together to the centre of 69 *a*. The distal ends of each pair are united as shown in *b*. Thus each united pair makes a complete ring by union of its distal ends. One of these rings is seen in chromosome 2 of 69 *b*. The lower focus of the middle (upper focus shown in *a*) forms the right side and the upper focus of the united ends forms the left side of this ring as shown in the illustration. Figure 69 *c* is an optical cross section of the spindle fibers; 69 *d* is the upper focus of the lower nucleus; and 69 *e* its lower focus. The corresponding chromosomes are again shown by the numbers. This nucleus has been rotated so that its chromosomes are not at right angles to those of the upper nucleus. The diffusion process has progressed farther, so that the chromosomes are very thin. They meet at the center of *e*, corresponding to the condition seen at *a*. The more distal part of the chromosomes is at *d*, and the sister monads of chromosome 3-3¹ seem to be separated. The distal ends of each are united, however, as in the upper nucleus, so that in this case also each chromosome forms a complete ring.

• A side view of this stage is shown in figure 70 and a little later stage in 71. In figure 72 the daughter nuclei have become more nearly round and a small granule is seen at the distal end of each. The chromosomes are still heavily stained at the proximal ends.

In succeeding stages, as shown in figures 73, 74, and 75, the chromosomes become very diffuse, but do not entirely disappear. The granule becomes more conspicuous and a second smaller granule appears near it. These granules undoubtedly correspond to the nucleoli of the spermatogonia and the first spermatocytes. They will be called the larger and the smaller nucleoli.

The spindle fibers of the first spermatocyte telophase are at first widely separated, (figs. 68, 69, and 70). They gradually draw nearer together and appear very black. A constriction may be seen at their middles so that they often look like a shock of wheat (fig. 74). At a later stage they disappear. Black bodies are seen at the time of the dissolution of the spindle fibers, as shown in figures 73, 74, and 75.

The telophase nuclei become the interkinesis nuclei of the second spermatocytes.

d. Second spermatocytes

1. *Interkinesis, prophases, metaphases, anaphases, and telophases.* The young nucleus of the second spermatocyte appears as shown in figure 76. The chromosomes are diffuse and are connected with the two very dense nucleoli, one of which is always larger than the other. The larger nucleolus becomes diffuse (fig. 77) and disappears (fig. 78). Meanwhile the chromosomes thicken (fig. 79), and the smaller nucleolus, still densely staining, appears attached to the end of a diffuse chromatic thread. The small nucleolus then divides (fig. 80), as the chromosomes become very thick.

The separate pairs have not been clearly distinguished at this stage, but it seems probable that the small nucleolus is descended from the pair of terminal granules of spermatogonia and first spermatocytes. Evidence for this is shown in figure 79, where the small pair of chromosomes is separate from the other two at the top of the figure. The small nucleolus is therefore associated with the larger chromosomes.

As the chromosomes become condensed, the small nucleolus can no longer be distinguished and the three pairs appear as in figure 81. The small pair in this and the succeeding figures is lettered *b*. One member of pair *a* in figure 81 is foreshortened as a result of twisting. In succeeding stages the middles of the members of each pair touch and the ends radiate somewhat at right angles (figs. 82, 83, 84, and 85). Thus three crosses are formed which tend to occupy a peripheral position in the nucleus. The monads do not cross each other, but merely touch, so that the opposite arms of the cross are parts of different monads.

Figures 83, 84, and 85 are from picro-formol-acetic fixation and, as a result, the chromosomes are somewhat distorted. Figure 83 shows a trace of the nucleolus between the larger pairs. It is surprising that this should persist so late in this cell as it usually disappears before this time. The cross-shaped dyads next group themselves into a metaphase, the ends of the monads

point toward the poles to which they are to go, and the middles still touch at the equator of the spindle (fig. 86). The fibers attach, as in previous stages, to the middles of the chromosomes.

As the chromosomes pull apart in anaphase (fig. 87), the ends tend to reverse their direction and to point toward the equator of the spindle. A later anaphase is shown in figure 88.

In the telophase (fig. 89) the chromosomes become diffuse, especially at their distal ends. In figure 89 the spindle fibers have been pushed out of place and the nuclei are at right angles to each other. At *a* the nucleus is shown in side view. At *b* is an optical transverse section. The chromosomes are plainly visible, but I was unable to count them on account of the small size of the nuclei. The telophase nuclei of the second spermatocytes become the nuclei of the spermatids.

e. Spermatids

The spermatid nuclei rapidly increase in size and the two nucleoli of unequal size again appear, with the diffuse chromosomes radiating from them (fig. 90). As the spermatid is changing into a spermatozoon, the chromosomes become more and more tenuous and the nucleoli become diffuse (figs. 91 and 92). One nucleolus can be seen after the other has disappeared (fig. 93). Figures 91, 92, and 93 are from a slide more darkly stained than figure 90. In figures 94 and 95 the staining is light and thus the spermatids do not show their chromosomes. The spermatid in figure 95 has begun to lengthen and is larger than that in 90. I judge it to be at a stage between figures 90 and 91.

f. 'Pathological' cells

Among the second spermatocytes and the spermatids cells are frequently seen which are evidently first spermatocytes which have failed to divide while the tetrads have divided. The division of the tetrad into its four monads is sometimes normal as in figure 94, but more frequently abnormal. In figure 95, *a* and *b* have each divided into four monads. Tetrad *c* has formed a cross, the longer arms of which have broken off, and one of

these has divided,—*c'*. In other cases the division is so irregular that it is impossible to determine the origin of the different elements. This karyokinesis without cytokinesis occurs very frequently in some individuals, not at all in others. When the division of the tetrads is normal, we thus have a cell with twelve chromosomes.

A type of cell, rarely found by me, corresponds to the pycnotic type of degeneration described by Lomen ('14). The nuclear matter seems very much condensed and the cytoplasmic area is very clear. These cells occur in various places in the testes of a few individuals and usually near the walls. Figure 96 is a drawing of two of these.

Another type of degeneration mentioned by Lomen ('14) and observed in my material is much more frequent than the pycnotic. In these cells the nuclei become very large and take a blue-black stain with iron-haematoxylin. They seem to absorb all the cytoplasm and to swell up to many times the size of the original cells. Very dark masses may be seen in these nuclei. Cells of this sort occur among the spermatogonia and they retain their form without disintegration down among the spermatids. They are more frequent in some individuals and do not occur at all in others. Figure 97 shows such a cell accompanied by a first spermatocyte nucleus lying alongside it and at a lower focus. The difference in the size and the staining-reaction of the two is very striking. The first spermatocyte shows its nucleolus containing four darkly stained areas, as is usual.

B. OVARIAN MITOSES

The ovogonial chromosomes appear in all essentials like the spermatogonial. Likewise the mitoses of the follicular cells of the ovary are not essentially different. Late prophases show three pairs, (figs. 98 and 99) one pair, *b*, smaller than the other two, *a* and *c*. One of the larger pairs, *c*, frequently shows two granules at one end. These may be comparable to the terminal granules of spermatogenesis or they may be sex elements. They are somewhat larger than the terminal granules usually are, but

the latter vary so much, both in size and in staining reaction, that I have been unable thus far to reach any decision regarding their character.

In one ovary two cells in late prophase were found which had the chromosomes split longitudinally. The small pair from one cell with each member split is shown in figure 100. The three pairs from the other cell are given in figure 101. This condition seems to indicate the double nature of each of these chromosomes at this stage, although the separation of the elements is probably the result of technique.

Figures 102 and 103 show metaphases in stage A. In the latter cell were counted as many as eight diffuse bodies, resembling the larger nucleolus, but distributed about in the cytoplasm near the chromosomes. In figure 104 the chromosomes are beginning to assume the parallel arrangement of stage B shown in polar view by figure 105. An equatorial view of the same stage along the parallel axis is shown in figure 106.

Figure 107 shows an early, and figure 108 a later anaphase, from two sections. The separate elements are not distinguishable at pole *a* of figure 108, but pole *b* shows six V-shaped chromosomes lying parallel to each other. Figure 109 is an anaphase with the members of the pair to the right fused. The chromosomes are approximately parallel, showing that they have been pulled out from the parallel arrangement of the metaphase plate. In figure 110 the chromosomes are crowded at pole *a*, separate and parallel at pole *b*. Figures 107, 108, 109, and 110 are from picro-formol-acetic fixation, which accounts for the fusion and the contraction of the chromosomes. Figure 111 shows an oblique view of an anaphase from two sections. An optical section is drawn at pole *a*, while the six chromosomes are shown entire at pole *b*.

Figures 98 to 106 are ovogonial; figures 107 and 111 are follicular; while the nature of the tissue from which figures 108, 109, and 110 were drawn is doubtful.

C. MITOSES OF THE THORACIC HYPODERMIS

The developing limb buds and wing buds of the thoracic hypodermis of the larvae show numerous mitotic figures. Three pairs of chromosomes appear in late prophase (figs. 112 and 113), one pair, *b*, smaller than the other two, *a* and *c*. Metaphases stage A, (figs. 114 and 115) and stage B (figs. 116 and 117) are similar to the corresponding stages of spermatogonial and ovarian mitoses. Figure 118 is a side view of a chromosome splitting in early anaphase and figure 119 shows the six chromosomes lying parallel to each other as they go towards the poles. The chromosomes in figure 119 are V-shaped but only one arm of each V can be shown. The relatively greater tendency of the chromosomes to appear fused in the drawings from hypodermal cells is due to the fact that fixation is interfered with by the chitinous part of the integument. This will be discussed more fully under Technique. More detailed description of the somatic complexes will appear in papers by Mr. Hance and Miss Holt.

D. SUMMARY OF OBSERVATIONS

The chromosomes of *Culex pipiens* are six in number in all diploid mitoses. At all stages the homologues tend to be approximated in three pairs. One pair is smaller than the other two.

As the chromosomes of diploid cells are passing from prophase to anaphase, they occupy a flat metaphase plate. Two successive stages, A and B, are distinguished in the metaphase. In stage A the chromosomes are bent rods, swollen at the ends and constricted in the middle. The middles tend to be nearer than the ends to the centre of the plate.

In stage B the chromosomes tend to be of more uniform diameter. The pairs assume an arrangement parallel to each other, which is continued into the anaphase. Division of each chromosome begins at the middle and the daughters pass to the poles as V's.

In the late prophase of the first spermatocyte the approximated pairs become crosses or rings. The chromatids of each

member of each pair separate at one or both ends and each unites with a chromatid from the other member. When this occurs at one end a cross is formed; when at both ends, a ring. A process comparable to this occurs at earlier stages in which the chromatids 'change partners' in part or all of their length. It is thus impossible to say whether the first spermatocyte division is reductional or equational.

In the second spermatocyte prophase the chromosomes appear as three pairs of rods, the members of which separate in anaphase. Three chromatids go to each spermatid.

The spindle fiber attachment is approximately at the middle of all chromosomes. In the tetrads it is attached to the middle of each chromatid.

A distinct nucleolus always appears between successive diploid mitoses and in the growth stages of the first spermatocyte.

It often shows four darkly stained regions. These nucleolar elements are separated at certain stages. Two of them appear to form terminal granules on one of the larger chromosome pairs, while the other two are connected with the middle of the same pair.

In the second spermatocyte interkinesis and in the spermatids, two nucleoli of constantly unequal size appear. In the second spermatocyte the smaller nucleolus becomes the terminal granules of one of the larger pairs, while the larger nucleolus becomes diffuse and disappears.

III. DISCUSSION

A. INTRODUCTORY

It will now be of interest to consider the causes for the wide difference of opinion prevailing among the investigators of *Culex pipiens*. This diversity is not merely a matter of interpretation: it concerns facts. The number of chromosomes, the normal occurrence of synizesis, the nature of synapsis, as well as the much discussed matter of chromosome individuality are all questions in regard to which the observations seem to differ.

B. TECHNIQUE

a. Experiments and observations

Among all of the causes of difference of observation and interpretation in cytology, the matter of technique is of prime importance.

The class in cytology under Dr. McClung undertook during the winter of 1914-15 to investigate *Culex pipiens*. The abdomens of larvae and pupae were cut off near the thorax and the tips of the abdomens also removed for better penetration. In some cases parts of the thoraces of larvae were also preserved. Division figures were found, not only in the gonads, but scattered through various parts of the somatic tissue as well. They were especially abundant in the developing leg and wing buds of the thorax, and were also found in the nervous system. In certain of the pupae the degenerating larval intestines also showed division figures, mostly multiple complexes.

The best fixation seemed to be given by a formula of Dr. Ezra Allen, consisting of saturated aqueous picric acid, 75 per cent; formol, 15 per cent; glacial acetic acid, 10 per cent. The results, however, were not very satisfactory, which seemed to be due in part to slow penetration, in part to the distorting action of the fixative. Flemming's fluid has been employed by Metz in his investigations on the Diptera. It does not give good results, however, unless the tissue to be fixed is dissected out in minute pieces and immediately placed in the fixative. An attempt to fix in Flemming without dissecting out the tissue resulted in shrunken and blackened sections, clearly the result of poor penetration and delayed fixation.

At the suggestion of Dr. Metz and Dr. Wenrich, therefore, in the fall of 1915, I dissected out the gonads of larvae and pupae in tap water and transferred immediately to strong Flemming. The gonads were drawn up in a capillary pipette and transferred to the fixative. After some experimenting it was found that the dissection may be done with greatest facility by cutting off the tip of the abdomen just posterior to the gonads and then pressing out the latter.

The gonads were fixed from two to twenty-four hours in small vials. The fluid was then replaced by water in which the tissue was left over-night. The water was then gradually replaced with alcohol until the percentage reached 95, in which the tissue was stained with eosin for the purpose of orientation in the paraffin. Clearing was done in aniline oil followed by bergamot or xylol and when all the water and alcohol were removed the gonads were transferred from the vials to melted paraffin. About one hour sufficed for thorough infiltration. Sections were cut 6μ in thickness and Heidenhain's iron-haematoxylin was used with no counterstain. A few testes were dissected out and fixed in Allen's picro-formol-acetic. This gave fair fixation but the chromosomes appeared much shorter and thicker and had a greater tendency to fuse. A few abdomens of adult females were torn to pieces in Flemming's fluid and good fixation of dividing figures was obtained.

It was found to be difficult to obtain good penetration of thoracic hypodermis. In the larvae this tissue is full of division figures, but the chitinous epidermis, binding so closely on the outside, and the adipose tissue, adhering on the inside, seem to prevent the proper penetration. Probably the fat takes up the osmic acid, as it appears much blackened. The attempt was made to remove the epidermis, and although the outer layer was removed, the inner layer still interfered considerably with penetration. By considerable searching, however, it was possible to obtain a few good figures at the edge of the tissue. Mitoses nearer the center were usually much distorted, and the chromosomes lying near each other were fused.

All degrees of fusion were observed in the chromosomes, corresponding to the distance of the cells from the cut edge of the tissue. This fusion is very striking in picro-formol-acetic. Often each pair appears like one chromosome, so that there will be only three chromosomes instead of six. In tissue put up in picro-formol-acetic without dissecting, spermatogonial pre-metaphases often appear so distorted that they fuse into a triangle, each corner of which corresponds to the approximated ends of two pairs. The chromatin may then become aggregated

at the corners so that three masses are formed, each connected to each of the others by two strands, corresponding apparently to the middles of the original chromosomes. These strands appear at times to have been broken and the three masses to have rounded off into three smooth round 'chromosomes.' All sorts of irregularities of the process, as would be expected, occur. The prophase chromosomes assume all sorts of irregular shapes and the entire contents of the nucleus may be shrunk into a 'synizetic' mass.

In gonads dissected out and fixed in Flemming the fixation is not by any means at all times perfect. This may be judged in general by the appearance of the tissue as a whole, whether shrunk or not. In some cases, however, the chromosomes appear well fixed, while the tissue may exhibit some degree of shrinkage. Figures 17, 18, and 19 are from one slide in which the general fixation was not good. The homologues are more or less shrunk and appear to touch each other. Figures 16, 20, and 21 exhibit the same stage with good fixation. The chromosomes are well separated.

Under normal conditions it is questionable whether the chromosomes ever touch each other during mitosis. The parallel union of the members of pairs in first spermatocyte telophase may be an artifact (figs. 68 and 69). In the early telophase of the spermatogonia (fig. 28), the homologues remain distinct.

The chromatin of the mosquito seems at times to have a tendency to flow together. In the cross-shaped tetrads the strands connecting the arms about the opening at the centre would be expected to be of approximately equal thickness—the width of a chromatid. Occasionally one or more of these strands may be reduced to a mere shred, even in prophase, before we would expect any tension upon it. Such a condition is shown by tetrad *b* of figure 54.

The contrast between the sharp outlines of the Flemming material (fig. 82) and the picro-formol-acetic fixation of the same stage (figs. 83, 84, and 85) is obvious. In this case the gonads fixed in the latter were dissected out before fixation. The conditions are therefore much better than in those not dis-

sected, but 'synizesis,' nevertheless, occurred. The fusion of the chromosomes in the picro-formol-acetic material is seen in figures 107, 108, 109, and 110.

b. Review and criticism of literature

It will now be of interest to consider the technique employed by the different investigators in the study of the chromosomes of Diptera.

Stevens ('09) "removed the anterior segments and secured good fixation in Flemming's fluid and fairly good in Gilson's mercurio-nitric." Most of her work was done, however, with aceto-carminic which tends to swell the tissue. The gonads were dissected out for the aceto-carminic method. The errors in Stevens' work are few and her results more consistent than either Taylor's or Lomen's.

Lomen ('14) simply cut the larvae, pupae, and adults in the middle and fixed in from 8 to 9 parts corrosive sublimate, 3 to 4 parts absolute alcohol. To 96 parts of this were added 4 parts of glacial acetic acid. This was called S-A-E. Zenker's potassium-bichromate, sublimate, acetic, K-B-S-E, was also used and was considered better for dividing figures. Lomen's figures are all very diagrammatic.

Taylor ('14) tried aceto-carminic for which she dissected out the gonads. She states, however, that it is not permanent, not good for finer details, nor for somatic mitoses. For material to be sectioned she used as fixatives, Benda's fluid, acetic-bichromate, Gilson's mercurio-nitric, Flemming, and Gilson-Petrunkevitch. She does not state whether she dissected her material to be sectioned, but since she laid such stress on somatic mitoses, it is probable that she kept her tissue largely intact. Her figures are consistent with each other neither in number of chromosomes nor in their form. They suggest very careful drawings of poorly fixed material.

Metz ('14) has made use of aceto-carminic preparations and Flemming material. In all cases the gonads were dissected out before fixation. The chromosomes stand out clearly and are

separate from each other except in figures 9, 15, and 16 of 'metaphases' in which the homologues are in contact. This 'synapsis' as Metz calls it, I believe to be an artifact.

C. SYNIZESIS

The word synizesis was coined by McClung ('05) to denote a "condition of the nucleus in which the chromatin is found massed at one side of the vesicle, without regard to whether it is a normal phenomenon or not." Synizesis is supposed usually to take place at the beginning of the growth period. The word synapsis has been used by many writers to denote this condition, but it is clear that Moore ('95), who coined the latter term, intended it to be used for the pairing of the chromosomes before reduction. This will be treated more fully under Synapsis.

The existence of synizesis, as a normal phenomenon, is questioned by many. In the case of the eggs of *Paludina*, however, Popoff ('08) observed it, and concluded, as a result of his measurements, that it is normal. There is no contraction of the chromatin, but rather a rapid increase of the nucleus in size, due to osmosis. This is not immediately compensated by expansion of the chromatin, and thus an appearance of synizesis is produced. Much the same phenomenon was observed by Lawson ('10) in plants and demonstrated by measurements.

Stevens observed synizesis in *Culex* ('09) and states that "the granular and beaded chromatin threads are wound about a large nucleolus." She considers that it bears no relation to synapsis but that it is a period of reconstruction. In *Musca domestica* ('08), however, she states that it does not occur.

Lomen ('14) is very skeptical of its being a normal phenomenon since he obtained it with certain fixatives but not at all with others. K-B-S-E, he states, gave it, but not S-A-E.

Taylor ('14) seems to contradict herself in her statements about synizesis. She considers that it marks off the spermatogonia from the first spermatocytes. When she finds it in very young larvae she supposes that they are precocious in maturation, but when she finds it in somatic cells (p. 391) she concludes

that it is not significant in *Culex* as it is in other creatures. Finally, (p. 394), it is concluded that synizesis represents an inactive phase of the nucleus. This last statement is probably true in a different sense from what Taylor intended.

In the Hemiptera Wilson ('12) observed a distinct synizesis occurring at the time of synapsis and is inclined to consider it a normal phenomenon. In speaking of "the polarized amphitene, or 'bouquet-stage,' that is characteristic of *Tomopteris*, *Batrachoseps* and other forms," Wilson states that it is "entirely wanting in these insects, where, in its place, we find the closely convoluted and apparently non-polarized synaptic knot, or synizesis. The controversy as to whether the latter is an artifact, due to the coagulating effect of the reagents seems to be terminated by the fact . . . that the synizesis may be seen in the fresh (living?) material immediately after gentle teasing apart of the cells in a normal fluid (Ringer's solution) in which the spermatozoa continue actively to swim." Wilson seems to question just how much alive "the fresh (living?) material" may be, and might also have questioned the perfect normality of Ringer's solution. It would seem that during the synaptic phase the chromatic elements are in a very unstable condition, and that any shock is likely to cause them to clump together.

Whatever may be the normal condition in other forms, I am convinced from observations with different methods of technique, that in *Culex pipiens* synizesis is an artifact.

D. CONTINUITY OF THE CHROMOSOMES

The matter of chromosome continuity has been ardently assailed by some investigators and as ardently defended by others.

It is tacitly held for *Culex pipiens* by Stevens and Taylor. Lomen ('14) considers the matter at length and concludes that all appearances are against it. He has, in first spermatocyte growth stage, a 'Brockenstadium' in which the chromosomes are completely broken up. The chromatin appears as fine granules

which collect into threads at a comparatively late stage. A comparable condition occurs between successive diploid mitoses.

I have not been able to count the number of chromosomes in all stages between telophases and early prophases, but I have nothing at all suggesting such a 'Brockenstadium' as Lomen figures. Fine chromatic threads are visible in all 'resting' stages in my material. They occur in the earliest first spermatocyte nuclei (figs. 29, 30, and 31), as well as in all stages from first spermatocyte telophase to second spermatocyte prophase (figs. 69 to 80). I therefore conclude that the chromosomes are continuous between mitoses and that Lomen's 'Brockenstadium' and 'Verstäubungsprozess' are artifacts.

E. CHROMOSOME NUMBER

Stevens ('09) concludes that, in diploid mitoses of *Culex pipiens*, each of the six chromosomes divides longitudinally, and six go to each pole. Three is the reduced number appearing in both maturation division.

Taylor ('14) considers three as the somatic number. She suggests that there may be but one maturation division, which is equational. This, she recognizes, would involve the non-participation of one of the gametic nuclei in fertilization. She suggests, as an alternative, that the homologues have fused in her material, thus reducing the number. She accounts for her disagreement with Stevens by supposing that the latter worked on *Culex pungens*. Stevens has used the names *pungens* ('09) and *pipiens* ('11) interchangeably. They appear to be the same, both taxonomically and cytologically. Taylor considers that one member of each pair appearing in diploid metaphase goes to each pole. The homologues are thus interpreted by her as daughter chromosomes.

Lomen ('14) has three chromosomes going to each pole in all anaphases, and three chromosomes appearing in prophase. But his spermatogonial anaphase shown in his figure 10 could as well be interpreted as having six. The prophase chromosomes each split transversely to form the six metaphase chromosomes.

But the only evidence that Lomen has for the cross-splitting is a slight constriction and bending of the prophase chromosomes in the middle. He states that Dr. W. Stempel, his professor, is inclined to consider six as the normal number, and concludes that therefore reduction must take place in the growth period. This, however, is inconsistent with his account of spermatogonial anaphases which, he states, show only three, and therefore reduction would be unnecessary.

From what I have said in regard to the importance of employing the best technique, it will be evident, I think, that the inconsistencies of the results of Taylor and Lomen are largely due to imperfect technique.

In a brief paper on *Corethra plumicornis*, Dehorne ('14) concludes that three is the diploid as well as the haploid number of chromosomes in that species. Only one figure is shown, very diagrammatic, of a somatic prophase. Dehorne speculates about the possibility of parthenogenesis as an explanation of the reduced number. It seems probable to me that here, also, the close approximation of homologues is the true explanation of the condition, and that a refinement of technique would reveal this.

F. NUCLEOLI, HETEROCHROMOSOMES, AND CYTOPLASMIC BODIES

a. Introductory

The occurrence of condensed bodies in resting nuclei and of darkly stained bodies in the cytoplasm has been the cause of much confusion and speculation in the history of cytology. It must be confessed that but little progress has been made in regard to our knowledge of the cytoplasmic bodies. The nucleoli, however, are somewhat better understood. In general they bear a staining reaction reverse to the chromosomes. When the chromosomes stain darkly the nucleoli are very diffuse or absent (?). When the nucleoli come into prominence, the chromosomes become diffuse or seem to disappear. For this reason it has often been assumed that chromatic matter passed from chromosomes to the nucleoli and the reverse. But there is no more reason to suppose that this takes place than that

certain changes are going on in each that make them darkly-staining at one time, not at another. That the latter theory is preferable would seem to be the case in the chromosome-nucleoli. In many forms the unpaired or accessory chromosome appears condensed in the resting nucleus, but becomes diffuse in metaphase or anaphase. Other odd chromosomes or parts of chromosomes condense when the others are diffuse.

b. Heterochromosomes

Stevens ('11) found in *Anopheles* an unequal pair of chromosomes attached to the smaller pair of euchromosomes in spermatogonial metaphases and in first spermatocytes. In ovogonia the corresponding elements are each equal in size to the larger member of the unequal pair. In resting spermatogonia the condensed pair is associated with a plasmosome and in growth stages the two members can often be distinguished. They are condensed and attached to the still diffuse smaller pair of euchromosomes. The first maturation division separates the members. The interkinesis condition was not made out, but the dimorphism of the spermatids was declared evident, each having a single nucleolus, which might be either large or small.

Unequal *xy* pairs were found by Stevens ('08) in nine *Myodaria*, unassociated with the euchromosomes. The larger member of each pair was shown to be the sex chromosome by comparison with the female in *Musca*, *Calliphora*, *Sarcophaga*, *Scatophaga*, *Tetanocera*, and *Eristalis*, but not in *Lucilia* and *Phorbia*. It is probable that the same condition obtains in the latter two genera as well; but in *Drosophila* the reverse seems to be the case according to Bridges ('16). Stevens supposed that in *Drosophila* the larger bent member was the *x*-element.

Stevens ('09) has made considerable search for heterochromosomes in *Culex*. Especial attention was given to the smaller pair, as it seemed to condense at some times earlier than at others. I have found no evidence for this in my material. No inequality could be found by Stevens.

My own evidence in regard to heterochromosomes in *Culex* is not at all conclusive and applies to only a few of the spermato-

gonial metaphases and one prophase. By reason of the twisting and the irregularity of the chromosomes at this stage and because of the variable number of diffuse bodies of similar appearance it has thus far been impossible to gain conclusive evidence. If, however, as figures 12 and 14 seem to indicate, we have a diffuse body attaching to one member only of one of the large pairs, we have here a possible sex element and a condition comparable with that of *Ascaris* as shown by Herla ('95) and Frolowa ('13).

c. Nucleoli

The plasmosome of *Culex* was noticed by Stevens ('09) and was thought to absorb chromatin extruded by the spireme.

Lomen ('14) noticed a clear space about the nucleolus and concluded, therefore, that the spireme is not at all connected with the latter. He considers that the variations in form of the nucleolus are degenerative. He finds only one in second spermatocytes, but occasionally two in the spermatids.

I have nothing at all to offer as an explanation of the nucleolar structures in *Culex*, but I believe that they all arise from a single pair of chromosomes. In spermatogonia and first spermatocytes they never show more than four elements, two of which, the terminal granules, are attached to the ends, and the other two, the 'plasmatic' elements, are connected with the middles of each member of one of the large pairs. Whether this is the same pair that is associated with the 'sex-element,' I am unable to say. The greater size of the plasmatic elements in relation to the terminal granules, and the more precocious tendency of the former to become diffuse, lead me to identify them with the larger nucleolus of the interkinesis nucleus and of the spermatids, while the terminal granules evidently form the smaller nucleolus as shown in the figures.

G. METAPHASE—STAGES A AND B

The peculiar parallel arrangement of the pairs of chromosomes, as in stage B of the metaphase of *Culex*, has not been noticed, so far as I am aware, in any other form. There are

suggestions of it in some of the figures in other works upon Diptera, but it is nowhere else shown that the chromosomes begin their division only after assuming this arrangement. In diploid complexes of *Drosophila ampelophila*, Bridges ('16) has shown a splitting at the ends of the chromosomes. The spindle fiber is median, as in *Culex*, but the spermatogonial and ovogonial divisions begin by a split at the ends of the chromosomes and "the separation at the point of attachment does not take place until the elongation of the cell." If we may judge from the few figures given, the chromosomes which are splitting appear to take on a parallel arrangement. In spermatogonial divisions of *Culex* this split at the ends does not occur, but it does occur occasionally in the somatic cells. The division of the somatic chromosomes, however, seems to be from the middles, comparable with the divisions of spermatogonia and ovogonia. These matters are to be discussed more extensively in a paper by Mr. Hance.

It has been noticed that, in well fixed material, the condensed chromosomes act as if they repelled each other. In second spermatocyte late prophase (figs. 82, 83, 84, and 85,) and metaphases (fig. 86) the ends of the chromosomes are far apart, while their middles appear as if tied together by the spindle fibers. This condition may also be seen in the tendency of the outer members of the pairs in diploid metaphases to bend away from the inner members at their ends while their middles are more approximated, as if pulled together or toward the center of the metaphase plate by the spindle fibers. In spermatogonia (figs. 11, 12, 13 and 14), this is striking, and is even more so in the actual preparations, in which differences of vertical focus are evident.

As the chromosomes condense and stiffen, the tendency is for the pairs to assume a parallel arrangement apparently under the influence from the tension of the spindle fibers. Figure 14 looks very much as if pair *a* would be pulled about beneath *b* and *c* and come up between the two and parallel to them. This appears to be taking place in figure 15, in which I have lettered the pairs in a comparable manner. The result would be a parallel

arrangement as shown in the succeeding figures. In figure 16 this process is not completed, and the chromosomes are more irregularly bent. Figure 20 shows a typical case in which the rods are comparatively straight. The ends of pairs *b* and *c* are somewhat bent outward as if they were repelled from the other pair. There seems to be considerable tension on the middles of the chromosomes which are beginning to divide.

Whether this be the explanation of the assumption of the parallel arrangement of the chromosomes or not, it nevertheless remains true that this position assures the middles getting as near the center of the plate as possible while the chromosomes are stiff and lie in one plane.

H. PSEUDOSYNAPSIS, SYNAPSIS, AND HEREDITY

The term 'Synaptic Phase' was first used by Moore ('95) who speaks (p. 284) of "the Rest of Transformation (Synaptic Phase) between the First and Second Spermatogenetic Periods." He states (p. 276), "the cellular generations of spermat- or oogenesis before and after the numerical reduction of the chromosomes will be distinguished as those of the first and second spermat- or ovo-genetic series." Under the heading "the Rest of Transformation" (p. 284) Moore considers the entire period from the last spermatogonial division to the formation of the tetrads. "The nuclear threadwork again grows coarser and thicker, displaying at the same time a peculiar tendency to contract to one side of the nucleus, leaving a great clear space across which stretch numerous linen filaments. The contraction is not so marked when the cells have been preserved with osmic acid, nor on the outside of sections which have been preserved with Flemming's fluid, where the osmium has acted directly upon the cells." "Whether it exists in nature or not, the cells display at this period, and at no other, a remarkable tendency to have their chromatin contracted in consequence of some internal change which renders these nuclear figures diagnostic of the particular period in question."

It is obvious then that Moore questions the normality of the contraction, nor does he consider the contraction the essential or

important phenomenon of this period. It is merely 'diagnostic.' That the numerical reduction of the chromosomes is the essential feature appears from page 287. "There are thus, after the rest of transformation, only half as many chromosomes, i.e., separate chromatic masses, as there were before, and the halving of their number, being brought about while the nuclei are still at rest, is to that extent comparable to what is now known to go forward during the maturation of the reproductive elements of plants. I therefore propose the term Synaptic phase to denote the period at which this most important change appears in the morphological character of reproductive cells."

Again, in 1905, Farmer and Moore, consider the question of reduction. The term synapsis is derived from the Greek *συνάπτειν*, to fuse together, and is used to refer to the union of the homologous chromosomes rather than to the contraction of the entire chromatin content. "The synapsis represents that series of events which is concerned in causing the temporary union in pairs of pre-maiotic chromosomes, previously to their transverse separation and distribution, in their entirety, between two daughter nuclei." Synapsis takes place then in the first gametocyte nucleus, in the rest of transformation.

The terms 'maiosis' or 'maiotic phase,' from the Greek *μείωσις*, reduction, are used "to cover the whole series of nuclear changes included in the two divisions that were designated as Heterotype and Homotype by Flemming."

Farmer and Moore subscribe to telosynapsis and prereduction. In discussing the spermatogenesis of an Acridian, Wenrich ('16) demonstrates parasynapsis and reviews the work of other investigators upon other forms. In the Acridian the chromosomes enter the synaptic period as single leptotene threads. The homologues conjugate parasynaptically during the zygotene stage, and at the end of the growth period a secondary split at right angles to the original plane of separation becomes evident. Four chromatids are thus demonstrated which are the four elements of the tetrad.

It would seem in general, from the more recent work, that parasynapsis is at least the more usual mode of pseudoreduction

and that either maturation division may be reductional, depending upon the species or upon the particular chromosome. Wenrich has shown that a single chromosome may divide reductionally either in the first or in the second maturation division and that this is governed by chance. The possibility of determining this was due to a morphological dissimilarity of the homologues. He concludes, in general, however, (p. 105), "As to which of the two maturation divisions is equational and which is reductional, no absolute rule can be laid down. The evidence, however, points to the probability that generally chromosomes with terminal spindle fiber attachment are not separated from each other until the second division, while those that have a non-terminal attachment are separated in the first, and that consequently in the former the reduction occurs at the second division, in the latter at the first division."

In the work on Diptera the term synapsis is used with such a variety of meanings that the whole matter is quite bewildering.

Lomen ('14) uses the term in what he considers to be Moore's sense to denote a crowding together of the chromatin at the beginning of the growth period.

Taylor ('14) avoids all trouble by using Haecker's ('11) "word 'syndesis' for the conjugation of the chromosomes, and 'synzesis' for their clumping together."

Stevens ('09) considers that "in *Culex* it is quite certain that parasynapsis occurs in each cell generation of the germ cells in the telophase." She uses the term synapsis to apply to the first spermatocyte metaphase and states that there is here a prolonged parasynapsis ending with a telosynapsis before the chromosomes separate. This is clearly a misuse of the term.

Metz ('14) uses the term synapsis to denote the association of homologues in metaphase and anaphase of diploid mitoses. In a short review ('16) he states that he has found this pairing of homologous chromosomes in all diploid cells of seventy-five species of Diptera, from the lowest to the highest of the order, and beginning in the egg before cleavage is completed.

Overton ('09) has shown this approximation of homologues in somatic cells of plants and reviews the literature dealing with this matter.

This approximation of homologues in diploid cells is evidently something very different from synapsis in the true sense, which is restricted to the first gametocyte. In the former case there is no reduction in number of the chromosomes which appear for the next mitosis. In the latter case we have pseudoreduction which persists until they are separated either by the first or by the second maturation division. This was expressed by Stevens ('08 p. 373) who, however, uses the term synapsis for pairing in diploid cells. "In the spermatocyte we get complete synapsis and reduction; in the spermatogonium, only a foreshadowing of reduction, and abundant proof that synapsis is here a side-to-side pairing of homologous chromosomes."

I would suggest therefore that the term synapsis be retained for the uniting of the homologues in the first gametocyte, and that approximations or brief temporary unions in diploid cells be called 'pseudosynapsis.' The fundamental difference between pseudosynapsis and synapsis is that the former results in no permanent reduction, while the latter results in not merely a pseudoreduction, a reduction in number, but ultimately in a qualitative reduction, a separation of parental elements, which may not take place until the second maturation division.

The term 'synaptic phase' was employed by Moore to denote the rest of transformation. The conditions have now been more closely analysed (Wenrich, '16) and it is shown that the uniting of the chromosomes, synapsis, takes place at the zygotene stage and that the homologues are not permanently separated in some cases until the second maturation division. I therefore use the term 'synaptic condition' for the paired condition of the homologues, which, in most forms, extends from the time of synapsis in the zygotene stage to the final separation of the homologues in the anaphase of the first or the second maturation division. We have seen that in the Diptera a synaptic condition occurs in diploid cells also, but that this is not brought about by a true synapsis, since it results in no permanent numerical or qualitative reduction.

It is while the homologues are in the synaptic condition that the closest union of parental elements takes place and this seems

to be of considerable importance in heredity. In hybrids the development is often normal up to this time, apparently because the chromosomes from the different species remain distinct. But when the chromosomes try to unite in synapsis they are unable to do so in a normal manner and greater or less sterility results. This has been shown by Guyer ('00) in hybrid pigeons, by Federly ('13) in moths of the genus *Pygaera*, and by Wodse-dalek ('16) in the mule.

As has been pointed out by Metz ('16) there is a union of the homologues in Diptera before cleavage is completed. This may account for the sharp delimitation of species and the non-occurrence of hybrids in this group, for, if matings of different incipient species took place, it is possible that the early cleavage cells resulting might become pathological through failure of the chromosomes of different origin to unite properly in pseudosynapsis. Thus the species would be separated in their incipency and tend to diverge under the influence of this 'physiological isolation.' In the Lepidoptera pseudosynapsis has never been observed, and hybrids and species-intergrades are common, as appears from taxonomic work and especially from the work of Harrison and Doncaster ('14).

It is probably during the synaptic condition in the first gametocyte that factors brought in by homologous chromosomes have an opportunity to 'cross-over.' The synaptic conditions, in forms in which partial coupling occurs, have never been investigated. In plants a number of cases of partial coupling occurs and it appears that this is partial in the formation of both male and female gametes. Bridges ('14) has summarized some of this data from Bateson and from Gregory and shows that the linked factors of the sweet pea and of *Primula* may bear a linear relationship to each other. Castle and Wright ('15) also have shown that in the rat linkage is partial in both sexes. Sturtevant ('15) has pointed out that in the silk-worm moth, the experiments of Tanaka show that linkage is complete in the female, but partial in the male. The extensive work on *Drosophila* by Morgan and his students has shown that linkage

is absolute in the male but partial in the female, and that the coupled factors bear a linear relation to each other.

Both genetic and cytological studies show that in the Lepidoptera the female is digametic, the male monogametic, while the reverse condition obtains in the Diptera. This, however, seems to have nothing to do with partial coupling which occurs in both sexes of the rat.

Thus far results from *Primula* and sweet peas (Bridges '14) and extensive evidence from *Drosophila* show that where partial coupling of three pairs of factors occurs, the factors stand in linear relationship to each other and that they are scattered, as if by chance, along a line, not collected together into groups. Moreover the factors which are very closely coupled are still in linear relationship, so that it would seem necessary, as Morgan and his students have done, to suppose that there is a transverse break in a pair of chromosomes and a re-fusion in such a manner that the broken end of each of the original members is united to the broken end of the other.

This theory, known as the chiasmatype theory, was first advanced by Janssens ('09) who suggested that it might explain genetic results. He supposed that the early tetrads twisted spirally and that homologous chromatids touched each other, broke at the point of junction, which was called the chiasma, and fused at this point with each other. Thus in text figure 4, B and C would undergo a break and re-fusion, while A and D would remain intact.

Janssen's investigations were done upon the amphibian *Batrachoseps*. As Wilson ('12, p. 442) says, his theory "gives the only simple mechanical explanation thus far offered as to how such an orderly exchange of materials may be effected." He is inclined to give credence to Janssen's results; but some doubt about the matter has arisen through more recent work. It is most important to investigate the conditions in some form in which the mechanism of heredity in regard to partial coupling is known, or to investigate the mechanism of heredity in forms in which the synaptic conditions are better understood.

The 'change of partners' observed in tetrad stages may have no significance for heredity in the sex in which linkage is complete, but may be associated with more important conditions, such, possibly, as breaks and re-fusions in the female of *Drosophila*, the male of *Bombyx*, both sexes of the rat, and in the development both of pollen nuclei and of ova in *Primula* and *Lathyrus*.

Culex may be expected to give no crossing over of factors in the male, if we may generalize so widely from conditions in *Drosophila*, but *Culex* is a fly of relatively lower order taxonomically and somewhat nearer to the common stem of *Diptera* and *Lepidoptera*. We may perhaps expect to find some connecting link between *Drosophila*, in which linkage is complete in the male only, and *Bombyx* in which linkage is complete in the female only.

The cytological results in *Culex* thus far throw no light upon these questions.

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EXPLANATION OF PLATES

The drawings were made with a camera lucida, using a compensating ocular 20X and a Zeiss homogeneous immersion apochromatic 2.0 mm. objective. They were drawn at a magnification of 3000 from the specimens and enlarged to 6000. The drawings have been reduced one-third in reproduction, giving a final magnification of 4000 diameters.

The drawings are listed below to show which are taken from the same individual, the sex and stage of development of the individual, and the fixation.

INDIVIDUAL NO.	SEX	STAGE OF DEVELOPMENT	FIXATIVE	DRAWINGS NUMBER
1	♂	Larva	Flemming	1, 7, 10, 11, 12, 13, 27
2	♂	Pupa	Flemming	2, 29, 30, 31
3	♂	Pupa	Flemming	3, 4, 5, 6,
4	♂	Larva	Flemming	8, 9
5	♂	Pupa	Flemming	14
6	♂	Larva	Flemming	15
7	♂	Pupa	Flemming	16, 34, 35, 36, 39, 41
8	♂	Pupa	Flemming	20, 21, 22
9	♂	Pupa	Flemming	17, 18, 19, 23, 25
10	♂	Pupa	Flemming	24, 26, 32, 33, 90, 91, 92, 93
11	♂	Larva	Flemming	28
12	♂	Pupa	Flemming	37, 38, 42
13	♂	Pupa	Flemming	43, 45, 46, 47
14	♂	Pupa	Flemming	41, 71
15	♂	Pupa	Flemming	48, 49, 50, 51, 54, 76, 77, 78, 79, 80, 81, 86, 87, 88, 89, 95
16	♂	Pupa	Flemming	52, 61, 97
17	♂	Pupa	Flemming	53, 55, 56, 57, 58, 59, 60, 62, 64, 65, 94
18	♂	Pupa	Flemming	63
19	♂	Pupa	Flemming	66, 67
20	♂	Pupa	Flemming	68, 69, 70, 72, 73, 74, 75
21	♂	Pupa	Flemming	82
22	♂	Pupa	Picro-formol acetic	83, 84, 85
23	♀	Pupa	Flemming	100, 101
24	♀	Pupa	Flemming	103, 104
25	♀	Adult	Flemming	98, 99, 102, 105, 106, 111
26	♀	Adult	Picro-formol. acetic	107, 108, 110
27	♂	Larva	Flemming	115, 116
28	?	Larva	Flemming	112
29	♂	Larva	Flemming	113, 114, 119
30	♂	Larva	Flemming	117, 118.

PLATE 1

EXPLANATION OF FIGURES

1 to 10 Spermatogonial prophases, early and late. Figure 9 shows a possible sex element.

11 to 14 Spermatogonial metaphase. Stage A. Figures 12 and 14 show possible sex elements.

15 Transitional from Stage A to Stage B.

16 and 17 Spermatogonial metaphase. Stage B.

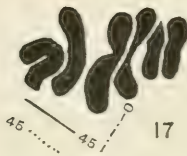
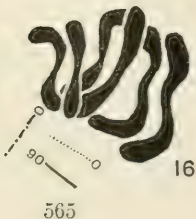
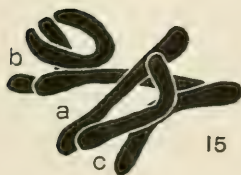
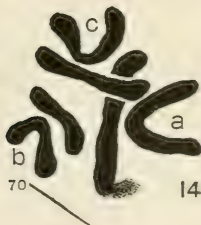
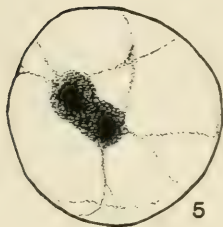
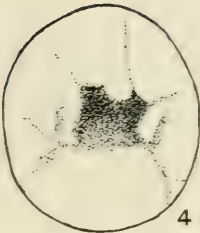
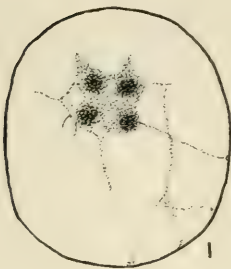


PLATE 2

EXPLANATION OF FIGURES

- 18 to 23 Spermatogonial metaphases.
- 24 to 27 Spermatogonial anaphases.
- 28 Spermatogonial telophase.
- 29 to 36 First spermatocyte growth stages.

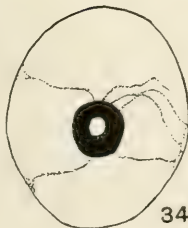
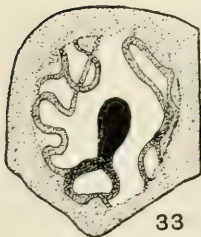
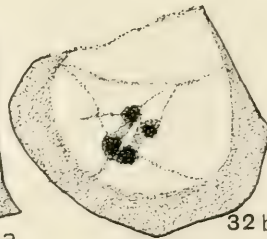
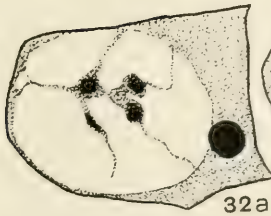
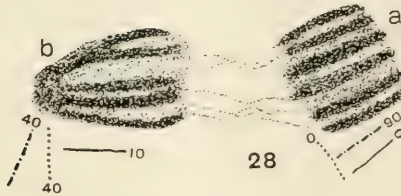
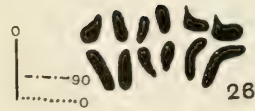
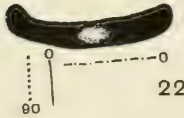


PLATE 3

EXPLANATION OF FIGURES

First spermatocyte prophases, showing nucleoli and terminal granules.

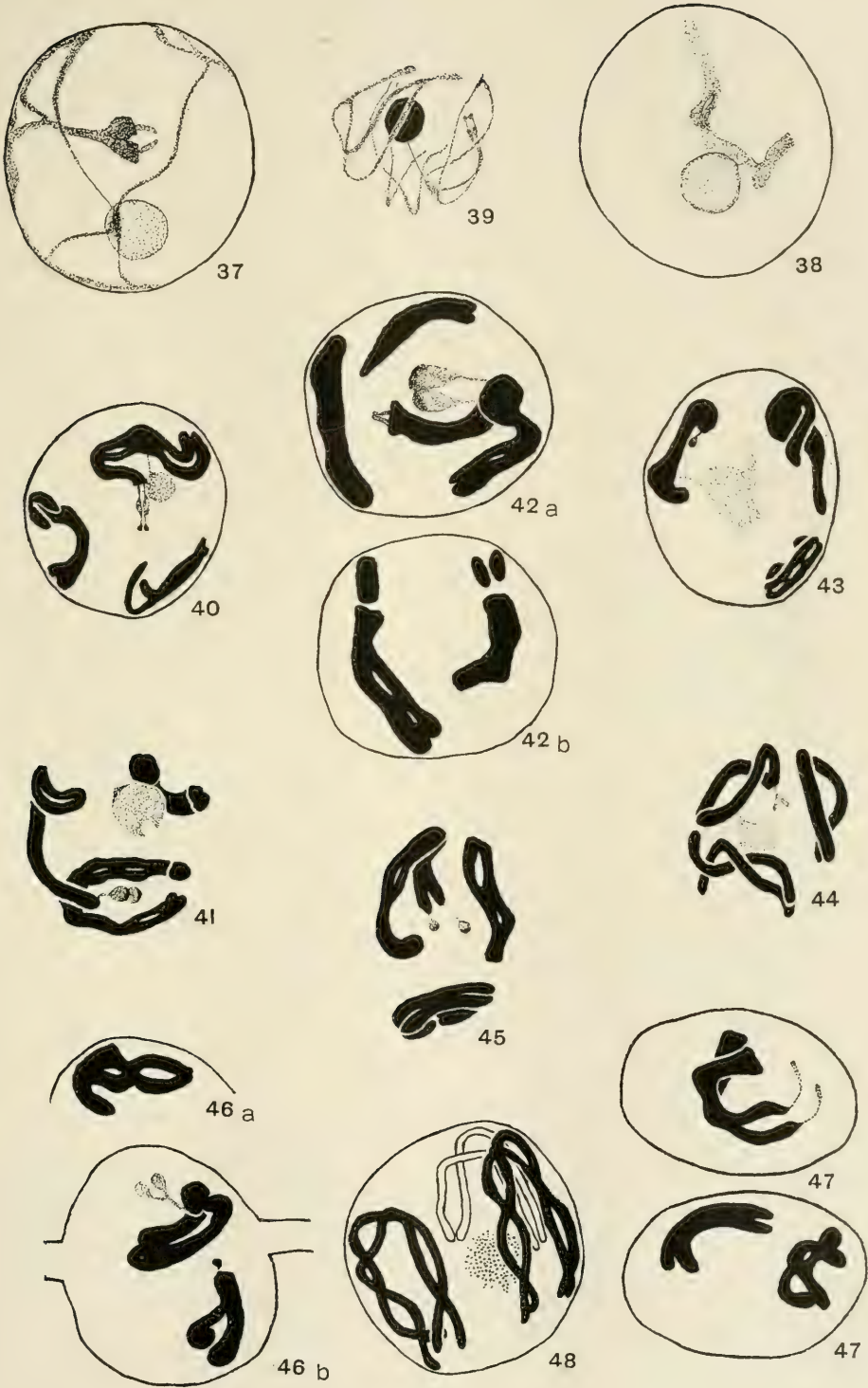


PLATE 4

EXPLANATION OF FIGURES

- 49 Prophase chromosome showing 'chiasma.'
- 50 to 64 Prophases, metaphases, and early anaphases of first spermatocytes.
The tetrads act independently of each other in passing through these stages.

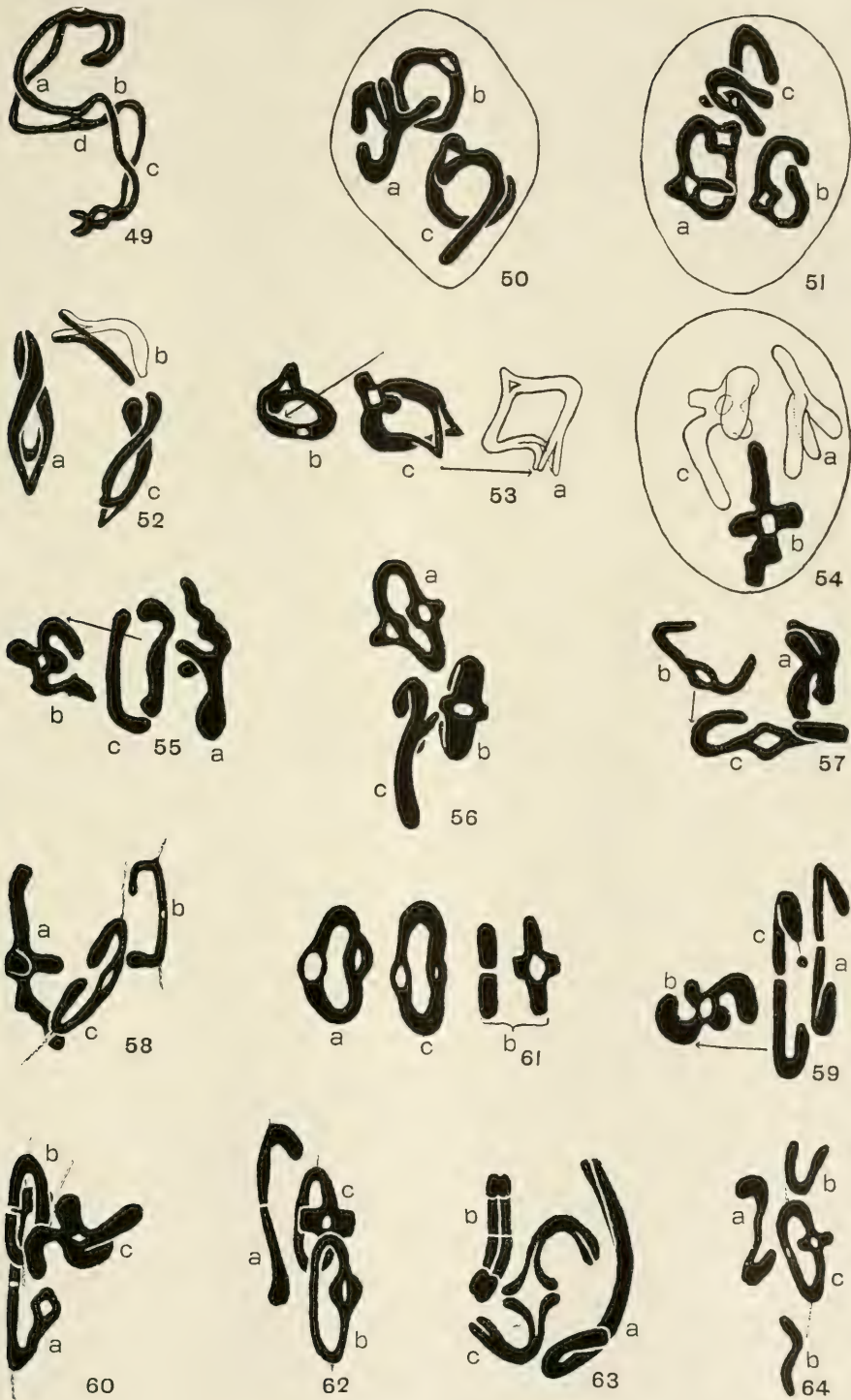


PLATE 5

EXPLANATION OF FIGURES

65 to 67 First spermatocyte late anaphases. Figure 65 is an oblique view. Figure 66 shows polar views of the two poles. Figure 67 is an optical section at one pole.

68 Very late anaphase of first spermatocyte. *a* and *c* show the chromosomes, while *b* is a cross section of the spindle fibers.

69 First spermatocyte telophases. *a* and *b* show the upper pole; *d* and *e*, the lower; *c* is a cross section of the spindle fibers.

70 to 75 First spermatocyte telophases.

76 to 79 Interkinesis nuclei showing nucleoli and chromosomes.

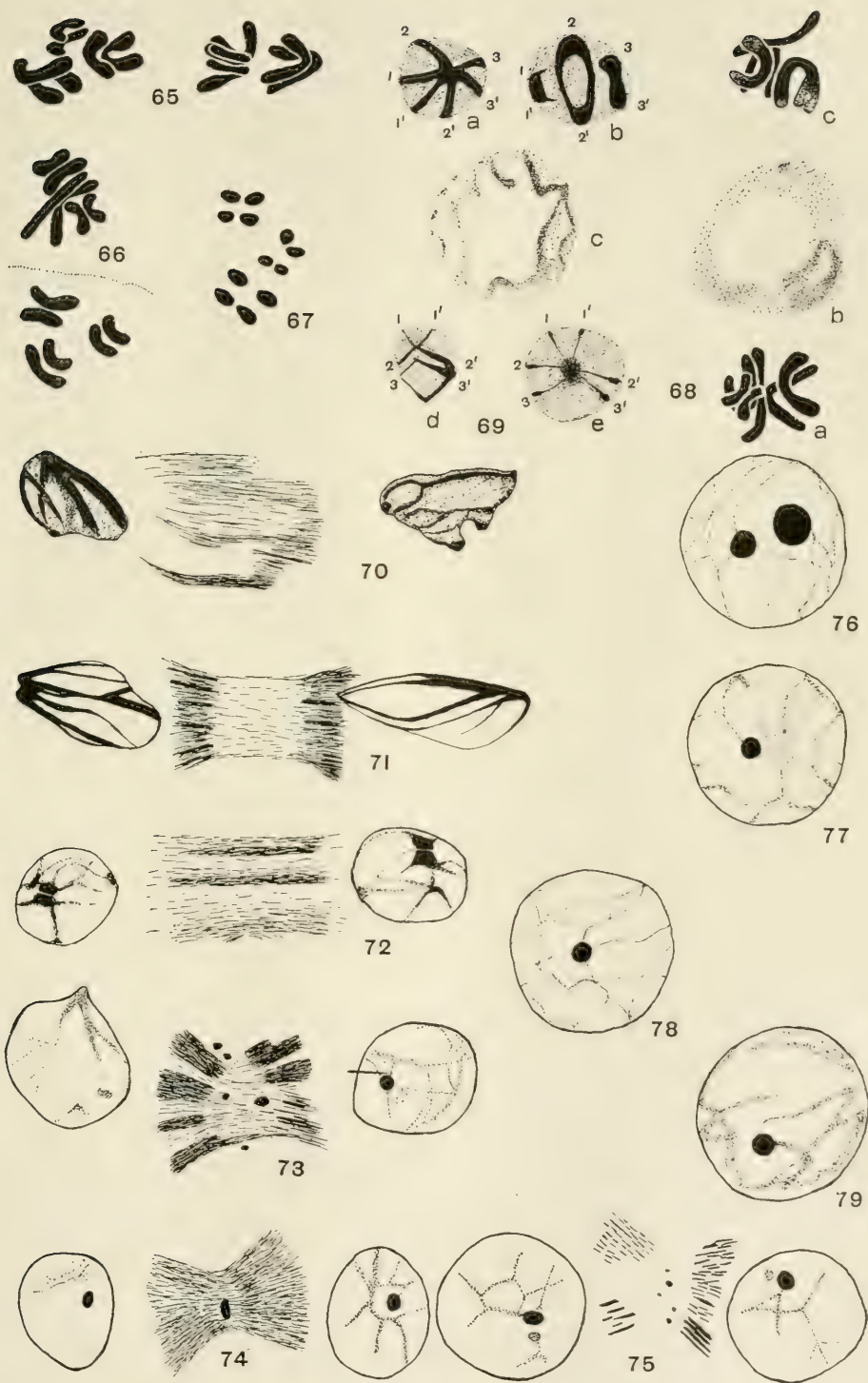


PLATE 6

EXPLANATION OF FIGURES

- 80 Interkinesis nucleus showing smaller nucleolus dividing.
- 81 to 85 Prophases of second spermatocyte showing three pairs of chromosomes.
- 86 Metaphase of second spermatocyte.
- 87 and 88 Anaphases of second spermatocyte.
- 89 Telophase of second spermatocyte.
- 90 to 93 Spermatids.
- 94 and 95 First spermatocyte cells showing karyokinesis without cytokinesis.
- 96 Pycnotic cells.
- 97 Pathological overgrown cell among the first spermatocytes.

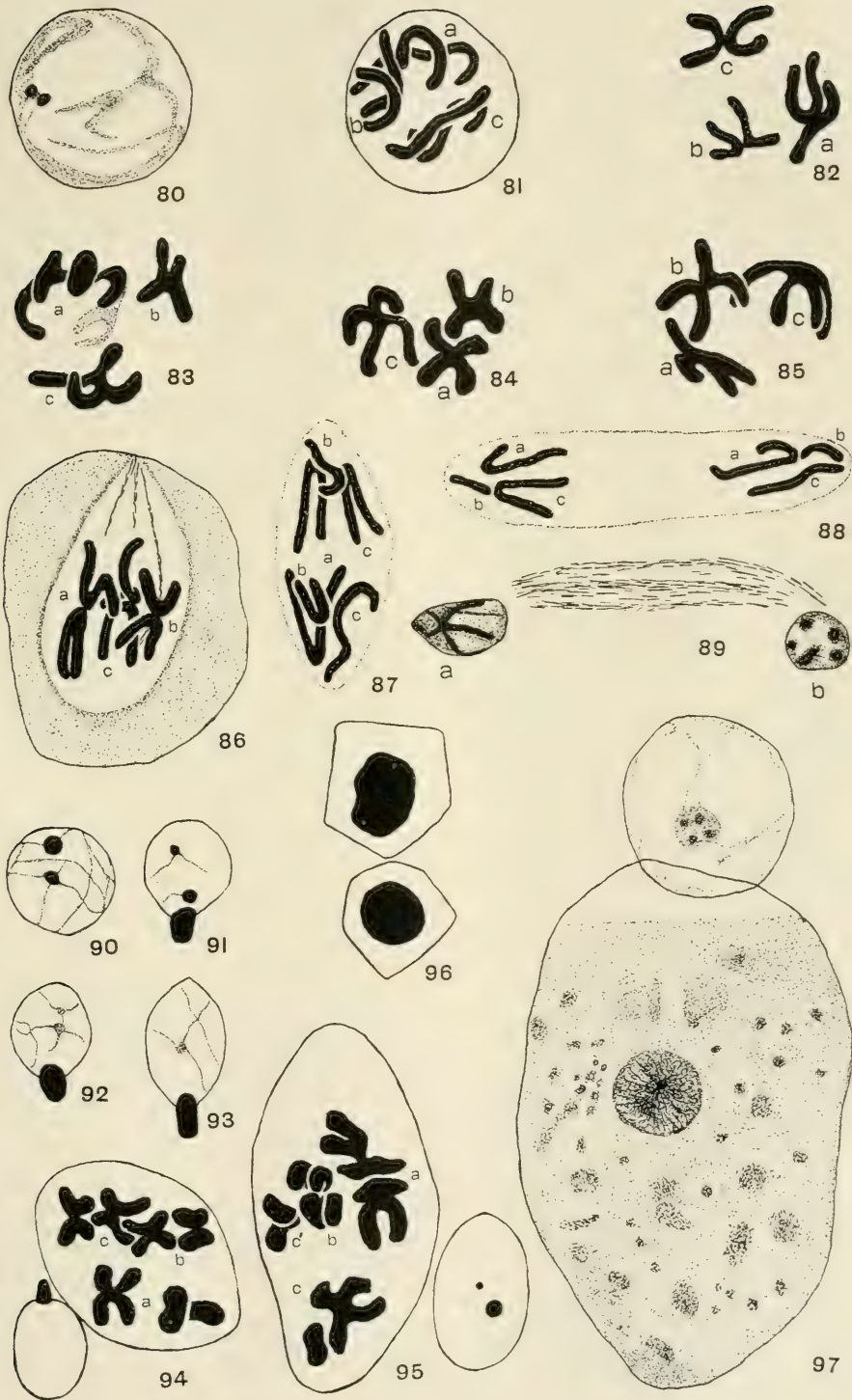


PLATE 7

EXPLANATION OF FIGURES

98 and 99 Ovarian prophases showing three pairs of chromosomes. The members are more or less fused. One of the pairs in each cell shows terminal granules (?).

100 Small pair showing each member split in unusual ovarian prophase.

101 The three prophase pairs showing each member split, from same section as figure 100.

102 to 104 Ovarian metaphases. Stage A.

105 and 106 Ovarian metaphases. Stage B.

107 to 111 Ovarian anaphases.

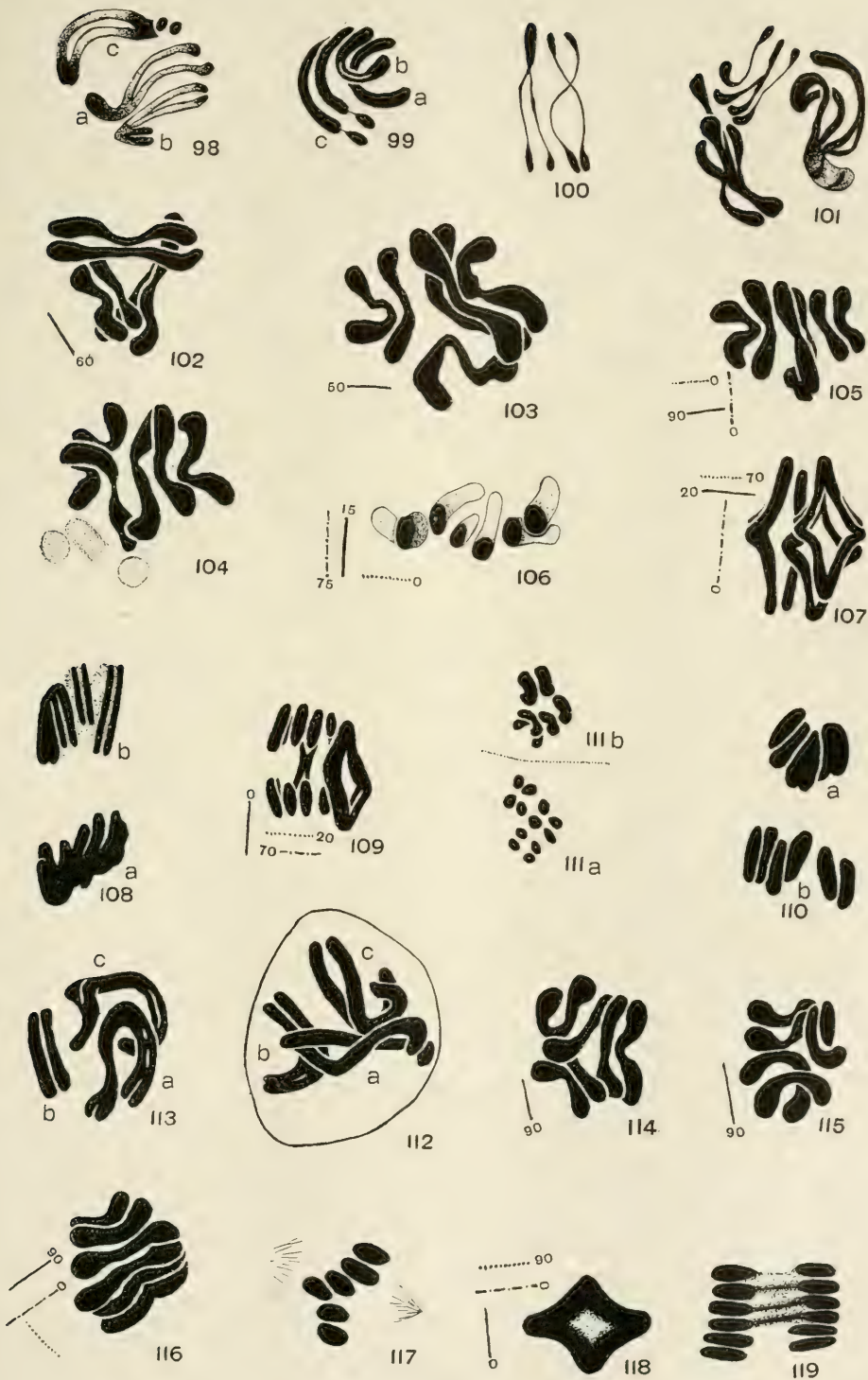
112 to 119 Somatic mitoses from thoracic hypodermal cells of larvae.

112 and 113 Prophases.

114 and 115 Metaphases. Stage A.

116 and 117 Metaphases. Stage B.

118 and 119 Anaphases.



THE SOMATIC MITOSES OF THE MOSQUITO CULEX PIPIENS

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TWO PLATES

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INTRODUCTION

The great mass of cytological work has been done on the germ cells, partly because of the abundance of mitotic stages found there, partly because of the greater ease of manipulating this tissue, but largely because of the great theoretical importance of the processes here visible. Beyond a few general sorties into the field of somatic cell division, which determined that the body chromosomes were usually present in the same number as in the reproductive cells, and were, in some instances, at least, similar morphologically, little has been done in the way of thoroughly working out the mitotic mechanism from prophase to telophase.

The ideal investigation into somatic cytology would follow the chromosomes of the fertilized egg as it evolved into the organism and as the cells differentiated into specialized tissues.

but unfortunately material favorable for the study of development is usually hopeless for cytological work, and the reverse is true. Some success which I have had with mosquito eggs leads me to believe that improved technique would make this insect a favorable form for such a study, but until such a technique has been developed, the study of the division figures found in the larvae of this form will prove, I think, both interesting and valuable. Because of an interest in somatic chromosomes and because of the contradictory reports that have been published on *Culex* this animal was selected for study. The results are complementary to Mr. Whiting's thesis on the spermatogenesis.

In this paper only what may be termed the normal somatic divisions will be considered, as Miss Holt has made a study of the multiple complexes.

It is a pleasure to take this opportunity of thanking Dr. McClung for the many facilities he has placed at my disposal and for his constant interest and advice. Discussions with my fellow workers Dr. Caroline Holt and Dr. P. W. Whiting have been of great assistance to me.

TECHNIQUE

An abundance of material was found in tubs of water standing in the greenhouses on the Campus. Larvae and pupae have been fixed, but only the larvae show any number of somatic divisions. The question of the method of fixation is an exceedingly important one, as the reasons for the various conclusions reached by other investigators of *Culex* chromosomes are quite evident from the results obtained with various fixatives. Eight different killing fluids were tried, each at three temperatures, 5°C., room temperature and 38°C. Of these the best has proved to be Flemming's solution to which had been added a little urea and used at room temperature. The urea was added to increase the rapidity of penetration. Another fluid which gave good results for some stages was what is designated in this Laboratory as P. F. A. No. 3 which was used at 38°C. The formula for this fluid is as follows.

	cc.
Picric acid saturated.....	75
Formalin	15
Glacial Acetic acid	10

Flemming's solution gave much the more accurate results particularly on the earlier stages. For perfect fixation with this fluid, however, the larvae must be teased finely in the solution or else the chitinous covering will prevent penetration. P. F. A. No 3 gives excellent results on the metaphase chromosomes but the early stages of the chromosomes are very likely to be wholly or partially fused, giving a totally erroneous impression of the actual conditions. When larvae were fixed in this killing fluid the head and thorax were severed from the abdomen and before placing in the warm fluid. Smears of the thorax were made and fixed in Flemming's solution but although thirty animals were prepared in this way no division figures were found. The general fixation was very good.

The material was dehydrated by allowing 95 per cent alcohol to displace the killing fluid drop by drop, until the fixative was entirely eliminated and the tissue stood in 95 per cent alcohol. When Flemming was used the material was first washed in water and then dehydrated in the same way. It was found unnecessary to remove the picric acid completely, as is usually advised following a picric acid fixative. The tissue fixed in P. F. A. No. 3 was cleared in aniline oil and the Flemming material was cleared in cedar oil. Either of these oils was followed with xylol, imbedded in paraffin and cut in sections six micra thick. In imbedding a number of thoraces or bodies the pieces were oriented one behind the other, which simplified the cutting materially (Hance, '15). When finely teased tissue was being prepared for cutting, the material in xylol was poured into a paper box or boat and the xylol was drained off through the paper, leaving the tissue massed at one end of the boat. The paraffin was poured into this paper form and the box was placed in melted paraffin. In this way it was easy to retain the finely divided tissue. Iron alum-haematoxylin was used exclusively in staining.

The methods of technique practiced here gave results consistent with others obtained in this Laboratory and are part of a number of experiments, employing the same reagents, under similar conditions.

OBSERVATIONS

Miss Taylor ('15) states that she had trouble in finding somatic mitoses, but I have had very little difficulty in locating active cells. The cells of the general tissues of the body are usually in a resting condition, but the limb buds in the thoracic region and the nervous tissue are frequently full of division figures. It is an interesting fact that the somatic divisions show some indication of rhythms of cell activity. The tissue may be entirely inactive or, when active, a large number of cells are taking part, and the majority of these cells will be in approximately the same stage of division.

I have never found any evidence of synizesis in either germ or somatic tissue, except in cases where the material was obviously very poorly fixed. While it seems evident that there must be some physiological (or probably only physical) peculiarity about the stage in which synizesis is observed which permits it to act differently to the other stages, nevertheless, I believe that the actual appearance of synizesis in fixed material to be an artefact.

Although most of the drawings have been made from mitoses found in the nervous tissue, similar stages have been found in most of the tissues of the body. These include nervous tissue, limb buds, intestine, epithelium, ovarian tissue and Malphigian tubules.

Prophase. The somatic cell in the resting or early prophase condition possesses a nucleolus. This disappears as the chromosome filaments appear. The earliest prophase stages I have found in active tissue are shown in figures 2 to 16. As can be seen there are three pairs of threads (two long pairs and one shorter pair) present, all more or less closely associated. The importance of the proper fixation in these stages can not be over-emphasized as I was misled for some time by finding (in the

P. F. A. No. 3 fixed material) only three threads with splits occasionally appearing at the ends of the threads. It is easy to understand how, unless the fluid reached the cell almost immediately, the delicate splits or lines of separation would be completely or partially obliterated. Such a case is shown in figure 1 where only three threads appear. It is very interesting to note the orientation of the three pairs of chromatin threads in the prophase. If the plates will be referred to it will be seen that the apices of the three V's in each cell (the point of spindle fiber attachment) are directed toward the same pole, suggesting the position in which the chromosomes lie when last seen in the anaphase. This condition is very suggestive from the point of view of the individuality hypothesis. As the prophases approach metaphase the paired chromatin threads seem to separate and gradually lose the characteristic prophase orientation. The beginning of this process is illustrated in figures 13 to 16.

I am unable to account, at present, for the size variation which occurs between the chromosomes of various cells. This will be apparent chiefly in the drawings of the prophase figures. Figure 7 is of an especially large cell. It will be noticed that the size relationship between the chromosomes of the same cell is apparently the same in all cases.

Metaphase. When the arrangement of the prophase is completely lost, the threads have shortened and thickened, have separated rather widely and the characteristic metaphase plate stage appears, figures 17 to 24, 26 to 30. This stage Whiting has designated as stage A. The chromosomes undergo another rearrangement and the three pairs line up side by side preparatory to division (Whiting's stage B), figures 25 and 31. A good side view of the spindle with the chromosomes distinctly visible is difficult to find but figures 32, 33 and 34 were drawn from clear cells in which the plates were slightly tilted permitting the six chromosomes on the spindle, each split preparatory to division, to be seen. Spindle fibers are visible but their actual attachment to the chromosomes is not evident. Judging from the anaphase figures, however, (fig. 37*a*) it seems evident that the fiber is attached to the apex of the V.

Anaphase. Clear anaphase figures are exceedingly difficult to find and the chromosomes are likely to be fused as in figures 38 and 39, giving a wrong impression of the real conditions. It would appear from these figures that three chromosomes are drawn to either pole, whereas in reality the drawings represent six chromosomes, as shown in figures 35, 36 and 37, the apices of whose V's have fused. In figures 35 and 36 six chromosomes are to be seen being drawn to either pole. Though these appear as rods they are in reality Vs, one arm of which is under the arm figured. In these cases the V-nature of the chromosomes can be determined by focusing. This condition is shown in figure 37a where one of the pairs has been twisted into the plane of the section. Figure 40 shows six chromosomes in anaphase from a polar view. There seems to be a tendency for the anaphase chromosomes of the somatic cells to turn slightly at right angles to the plane in which they separate, as Whiting has reported for the germ cells. In figure 36 the ends of the chromosomes are all tilted vertically to the plane of the paper. Whether this is due merely to the angle at which the arm of a V might lie or is a real change of axis, I am not certain.

Artefacts. In all stages of mitosis in poorly fixed material are to be found expressions of the faulty technique. A fairly complete gradation of fixation may be found which helps to indicate how other investigators (Seiler and Taylor) may have been led astray in their studies of the chromosomes of *Culex*. In cells so exceedingly small as these the minute separations would be very quickly obliterated if they were not immediately preserved by the killing agent. The fusion of the chromatin elements is a very common occurrence unless great care is taken to preserve the tissue properly, as I have shown in a paper to be published shortly on mammalian technique. (Hance '17.)

DISCUSSION OF RESULTS AND OF THE LITERATURE

The above account of the somatic mitoses has shown that there are six chromosomes in the body cells, corresponding in size and position to the six chromosomes of the spermatogonia found by Metz ('16) and Whiting (in this journal). In describ-

ing the ovogonial and spermatogonial chromsomes Miss Stevens ('10) has reported much the same cytological conditions in *Culex pipiens* as have been found by Metz and Whiting. "Each of the six chromosomes divides longitudinally, and the pairing of the daughter chromosomes probably occurs in the telophase, for very early prophase show the chromosomes paired and twisted together, forming three spireme threads which gradually shorten and separate for mitosis."

Miss Taylor reports three chromosomes for a number of somatic cells but fails to find any cells showing six. She figures a split appearing at the ends of some of the chromosomes, which condition I find in poorly fixed material. The split indicates the beginning of the line along which two chromosomes have fused. Her failure to find six may be due to imperfect fixation, as may be judged from her figures. The importance of proper fixation has already been emphasized.

In the case of *Culex pipiens* it is evident that when a spermatozoa with three chromosomes unites with an egg with three chromosomes the somatic number of six is produced. The comparatively little work which has been done on somatic mitosis has tended to show that the behavior of the somatic chromosomes may not always be the same as that of those of the germ cells. (Hoy '16.) By this I do not mean that the differences which seem to exist need in any way interfere with the current cytological hypotheses, although other writers seem to think so. It is quite evident that in *Culex pipiens* there is no difference between the two groups of cells. It will be of interest in this connection to review the chief literature dealing with the body chromosomes. A discussion of the results will not be attempted at present.

The breaking up of the chromosomes in the somatic cells of *Ascaris* is too well known to need further discussion.

In Morrill's work ('10) the drawings of the somatic chromosomes in *Archimerus alteratus*, *Anasa tristis* and *Chalinidea vittigera* are generally larger and longer than those of the reproductive cells. The relative proportions of the two sets of chromosomes appear to be the same, however. In an *Anasa tristis*

embryo possessing twenty-two chromosomes he found a cell with twenty-three chromosomes and in a twenty-one chromosome *Chalinidea* embryo a twenty-two chromosome cell was found. Morrill noted this difference in size and stated that the chromosomes of the embryo started out long and shortened as the embryo became older.

In Seiler's paper ('14-'15) on the cytology of *Lepidoptera* some figures of somatic chromosomes are quite noticeably larger than are the spermatogonial chromosomes. The number is the same.

Wieman ('13), in his very clear somatic figures taken from a human embryo, found the number of chromosomes to vary, and he pointed out that, "it is also known that the somatic mitoses do not always show a number identical with the premeiotic one." He reviews other similar cases which I have omitted.

In a paper on the Wandering Jew ('15) I called attention to the morphological difference between the somatic and the germinal chromosomes, the former being much more elongated. This may not be an entirely fair comparison, however, as the pollen eventually aborts and the behavior of the pollen chromosomes is pathological. In a study I have been making of the *Oenothera* mutant *scintillans* the somatic chromosomes have a wide range of number (from 15 to 21) in the cells of the same plant. It has been possible to show in this case, by special methods developed for the analysis of the chromosomes, that the 'extra' chromosomes are the product or result of fragmentation of the longer chromosomes, that the fragments divide regularly and that the total length of the chromosomes in the cells possessing extra chromosomes is the same as that of the type group. The relation of the somatic to the germinal chromosomes has been conclusively shown.

In his book, *Heredity and Environment*, Conklin ('15) has made a statement that would seem to sum up the suggestions of Wieman and which expresses my own belief. "Differentiations of cells are not due to the differentiations of their nuclei, but rather the reverse is true; such differentiations of nuclei as occur are due to differentiations of the cytoplasm in which they lie.

Nevertheless differentiations do not take place in the absence of nuclear material, and it seems probable that the interaction of nucleus and cytoplasm is necessary to the formation of the new cytoplasmic substances which appear in the course of development."

SUMMARY

1. Three pairs of chromatin threads appear in the prophase. The threads are V-shaped and the apices of the V's are directed toward the same pole suggesting the anaphase position (figs. 2 to 16).

2. In the metaphase the threads have shortened and thickened and have become more widely separated. There are three very definite pairs or four long and two shorter chromosomes (figs. 17 to 31).

3. Before the division begins the position of the chromosomes is altered once again and the chromosomes come to lie side by side (figs. 25 and 31).

4. The spindle fibers are apparently attached to the apices of the V's.

5. The somatic chromosome number is six, as is that of the spermatogonia and ovogonia.

6. The number is the same for all the tissue studied. These include nervous tissue, limb buds, intestine, epithelium, ovarian tissue and Malphigian tubules.

7. The importance of proper fixation has been emphasized.

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DESCRIPTION OF PLATES

All the figures are reproduced at a magnification of approximately $4500\times$. All the drawings were made from the Flemming preparations, except figure 29, which was taken from tissue fixed in P. F. A. No. 3. The drawings are all taken from nerve tissue except figure 29, which is from a limb bud.

PLATE 1

EXPLANATION OF FIGURES

- 1 Prophase showing only three threads. This cell is the product of poor fixation.
- 3 to 16 Prophase stages.
- 17 to 20 Metaphase stages, polar view.



PLATE 2

EXPLANATION OF FIGURES

- 21 to 31 Metaphase stages, polar view.
- 32 to 34 Metaphase stages, side view.
- 35 to 37 Anaphase stages, side view.
- 38 and 39 Poorly fixed anaphases.
- 40 Anaphase stage, polar view.



GERM CELLS OF COELENTERATES

III. AGLANTHA DIGITALIS

IV. HYBOCODON PROLIFER

GEORGE T. HARGITT

Zoological Laboratory, Syracuse University

III. AGLANTHA DIGITALIS

THIRTY-FIVE FIGURES (THREE PLATES)

STRUCTURE

Aglantha is a member of the order Trachomedusae, to which also belongs *Gonionemus* and other medusae. This order is usually characterized as having no hydroid or polyp stage and consequently not showing an alternation of generations, the egg developing directly into a medusa. Perkins ('02) has shown that in *Gonionemus* the egg forms a planula which becomes a small hydra-like organism which is sedentary and has the general form and habits of a polyp. From this polyp buds are produced and these, on being liberated, form other independent polyps. Perkins assumes the polyps to transform directly into medusae, after a short period of budding; this transformation was not actually followed. Since such a life history has been established for one member of the order, it may be that others display the same abbreviated and more or less transitory polyp stage. The life history of *Aglantha* is not known; it may have a polyp stage, or the egg may produce a medusa by direct development.

Aglantha has a tall, conical shaped bell with eight radial canals extending from the stomach to the ring canal on the margin of the bell. Gonads are attached to the radial canals close

to the stomach, but hang down freely into the bell in a sausage-like form, being fastened by only one end. Mayer ('07) says that "only female specimens have been observed;" probably this is merely a mistake in Mayer's description; both sexes are known, and the material collected for this study contained males and females in abundance. The medusae occur early in the spring, as a rule, the ones used for this study having been taken early in April at Woods Hole. For the systematic position of these medusae and for a fuller description of their structure reference should be made to systematic papers such as: A. Agassiz ('65), C. W. Hargitt ('04), Mayer ('07).

Each gonad of *Aglantha* contains a cavity which is in direct communication with the cavity of the adjacent radial canal; it is by this means that food is supplied to the gonads for the nourishment of the germ cells. In the ovaries there may be an absorption of the smaller oocytes by the growing eggs, but this is not marked nor abundant, nourishment comes chiefly from the food in the canals. The number of cells present in each ovary is very large, and as growth does not begin at the same time in all, a single ovary may show a great many stages in the oogenesis. Consequently the eggs mature at different times and are liberated from the ovary a few at a time. The eggs escape by rupturing the outer ectodermal covering of the gonad, though a few have been found in the cavity of the ovary from which they doubtless escape by a later rupture of the entire wall of the ovary.

Since the eggs mature at different times the breeding season extends over a longer period than in those *Hydrozoa* in which all eggs mature at one time; still the breeding season is limited to a few weeks or less. The entire process of growth and maturation is carried on and completed within the gonad, and the eggs have formed both polar bodies and are ready for fertilization as soon as they are liberated from the ovary. This is a fortunate circumstance for the study of the oogenesis; since the complete history of the changes passed through in the maturation process may be determined from a study of the gonads of the medusae, it is not necessary to collect liberated eggs. It is in these liber-

ated eggs that one would look for fertilization and cleavage; the difficulty of securing these eggs has resulted in leaving those phases of development out of consideration. Whether there is any periodicity in the time of liberation of eggs was also left undetermined.

The eggs are relatively rather small as compared with those of many Coelenterates, but the nucleus is relatively much larger than in any other coelenterate egg which has come under my observation. There is a sharpness and clear cut definition in the nucleus and its elements which renders this form peculiarly favorable for study. The chromatin masses are well defined and the chromosomes are very large and few in number, which makes their study easy. All in all, the egg of *Aglantha* is the most favorable of all the Hydrozoa which I have studied. Many stages of the spermatogenesis have been observed, but as these cells are small (though the nucleus is large) it is difficult to determine all phases of the process. Because of the incompleteness of the observations on the development of the male germ cells I have not included them here.

ORIGIN OF GERM CELLS

The material used for this study was abundant and covered the entire period of oogenesis except the very earliest stages. In the earliest medusae obtained the gonads were tiny spots scarcely noticeable without magnification; to secure earlier stages in oogenesis very immature specimens would be essential and these probably would be found before the time when medusae are abundant. In the youngest specimens obtained the tiny gonads had already passed the stage of primordial germ cells and a considerable number of oogonia or of spermatogonia were present. The method of formation of germ cells was therefore not determined, but their place of formation was quite clear.

The gonads are made up of an entodermal epithelium lining the cavity, a mass of germ cells and a thin ectodermal tissue covering the outside. In young gonads it is clear that they arise by an evagination of the radial canal to form the cavity of the gonad, and a proliferation of the ectoderm cells to form

the mass of the gonad which protrudes into the sub-umbrellar cavity. These proliferating ectodermal cells are the oogonia or spermatogonia of the gonads, for it is clearly shown in sections that there is practically no difference between the germ cells and the outer covering of the gonad. Indeed it is possible in almost any section of young gonads to see the ectodermal tissue of the outside of the gonad, and the mass of germ cells more or less intermingling. The cells of the radial canal and the cells of the entodermal lining of the cavity of the gonad are distinctly a gastrovascular tissue. They are long columns, with the cytoplasm much vacuolated, the nuclei are smaller and have an appearance different from those of the ectoderm and the germ cells. It is by a continued proliferation of the originally few germ cells that the large gonad filled with germ cells is produced.

OOGONIA

The proliferation of the original germ cells is rapid and there is produced an enormous mass of these cells. The divisions are mitotic in character, apparently without exception, and the proliferating period is more or less limited and regular, since a cessation of division takes place in all cells of the gonad about the same time. The ending of the division period is not abrupt, for a few mitoses may be found scattered through the oogonia after the process has apparently come to an end, but in a full-sized gonad filled with oogonia it is only by long search that a few divisions can be found. After growth has begun in some of the cells of the ovary there has never been found a case of dividing oogonia. The early stages in oogenesis are, therefore, quite regular and uniform; such variations as occur in older gonads are due to differences in the time at which the growth period began, with perhaps some possible variations in the rate of growth.

Figure 1 represents a portion of one side of an ovary and shows the long columnar epithelial cells of the gastro-vascular cavity, the mass of germ cells, and the ectodermal covering of the gonad. In this particular ovary oogonial divisions had ceased

and growth was just beginning in a few of the oocytes. The cells closer to the cavity of the gonad are oogonia, those in a zone toward the outside are oocytes; of the latter there are two in the figure which have grown to a considerable size. This arrangement of oogonia and oocytes in zones is characteristic of young gonads but is not observed after growth has begun.

The oogonia possess nuclei which occupy the greater portion of the cells, leaving only a narrow border of cytoplasm. The cytoplasm and the nucleus of such a cell are filled with an evenly distributed, finely granular, and close packed protoplasm which shows no particular structure. It makes no difference what fixation is used, the cytoplasm and nucleus always have this appearance and are so similar it is possible only with high powers of the microscope to clearly define the boundary line, and only with an oil immersion can a nuclear membrane be demonstrated. Within the nucleus is a very prominent, deeply staining nucleolus which appears to be largely chromatic in composition and may contain the greater portion of the chromatin of the nucleus. Figure 2 is an oogonium as seen under high magnification, with the nucleus represented as being a little too sharply defined. The impression one gets from a superficial examination of an ovary of this age (as figure 1 suggests fairly well) is that of cells with very small, sharply defined, deeply staining nuclei, which appear too small for germ cells. Close observation with high powers makes it clear that this body is a nucleolus which is within a large and ill-defined nucleus. The same appearance is characteristic of spermatogonia.

According to our knowledge of the behavior of germ cells in many animals we should expect to find the contraction phase (synizesis) of the chromatin, and the synapsis or conjugation stage of the germ cells occurring after the last oogonial division before, or just as, growth starts. There is no difficulty in determining which cells are oogonia and which oocytes after growth has started, and even in the younger gonads a difference in size is often observed. But even in the absence of marked size differentiation the different stages may be recognized by position, since the oogonia are grouped near the cavity of the gonad

while the oocytes occupy a zone toward the periphery, though after growth is under way this zonal arrangement is lost and the small cells are displaced by the pressure of the larger growing cells. Figures 5 and 6 show the last divisions of cells in the oogonial zone, the drawings having been made to show the equatorial plates of the metaphase of mitosis in which these cells were found. That these divisions are previous to synapsis is certain since they contain twice the number of chromosomes found in the polar bodies and pronuclei. These chromosomes number sixteen, vary a good deal in size, somewhat in shape, and show with remarkable clearness. If one attempt to match these chromosomes, i.e., seeks to identify pairs, there is some difficulty which results in disappointment. For instance, the smallest chromosome in each case (shown in the lower left portion of the chromosome groups in figures 5 and 6) is clearly without a mate of the same size. The one which, on account of size, would most logically appear to be the mate of the small chromosome is marked 'a' in figures 5 and 6. Such a pairing would involve more than the usual discrepancy in size between synaptic mates. Furthermore, I can not convince myself that the other chromosomes are clearly capable of being grouped into similar pairs, though they would agree more closely than the members of the smallest pair.

According to the usually accepted view there should be two chromosomes of a kind and their similarity should be so marked as to make a pairing of them fairly easy. What does the failure of these chromosomes to meet the expectation mean? Is it that chromosomes are not paired and synapsis does not occur; or is the conjugation between unlike chromosomes? The latter must be the case since there is clearly a reduction in number of chromosomes and therefore we have no reason to doubt that a synapsis, or its equivalent, takes place. The figures of the chromosomes of first maturation spindles in plate 3 show that the division of chromosomes in this mitosis is not always exactly equivalent. This would seem to give evidence that the synapsis may be between unlike chromosomes.

The search for synapsis stages was long, exhaustive, and not altogether satisfactory on account of the lack of success. Figures 3 and 4 may suggest synizeses or contraction phases, or they may be spireme stages of oogonial prophase. The size of the cells, approaching that of figure 2 and being smaller than the cells of figures 5 and 6, which are certainly oogonia, suggests the interpretation of this condition as an oogonial prophase. The same thing is indicated by the number of loops in figure 4; there were fourteen of these loops, so far as could be determined, and this is so close to the diploid number of chromosomes that it is probably sufficient warrant in itself for calling figure 4 a prophase of an ordinary oogonial mitosis and not a contraction phase. However, the arrangement of the chromatin in distinct loops suggests the bouquet stage of early maturation phenomena. The number of loops in such an interpretation would indicate that a synapsis had not yet taken place. The position occupied by these two cells (figs. 3 and 4) was on the border line between the oogonial and oocyte zones. It seems probable that the size of the cells is one of the most crucial bits of evidence and hence these figures are merely phases in an ordinary oogonial division. From this it follows that synapsis and synizesis stages are absent. That this is not due to the material is clear, for the search was diligent, the material abundant, the age of the gonads right for the occurrence of such stages.

From the conclusions of the above paragraph it follows that figure 7 is the stage which comes next after figure 2, for figures 3 to 6 are merely oogonia, one of which (fig. 4) simulates a synizesis stage. This seriation of stages is certain, as the size of the cells demonstrates, and it is the most obvious conclusion from a study of many gonads. When an entire gonad is under observation there are a large number of cells like figure 2 near the center of the gonad and many resembling figure 7 toward the outer side. If a careful and exhaustive search reveal nothing of synizesis or synapsis one might conclude that there is no definite and precise series of chromatin changes which leads to chromosome formation and reduction. Perhaps it would be better to say no visibly definite and precise changes, since there

are, without doubt, chemical changes occurring, probably also physical ones, and these would of course be more or less definite and precise, even though they might not be visible.

OOCYTES

From this period up to the completion of the maturation process there is no break and no uncertainty regarding the changes or the order in which they occur. From the condition in figure 2 up to the formation of the completely matured egg the changes are briefly as follows: there is a gradual vacuolation of the cytoplasm leading to the characteristic alveolar arrangement, and a gradual condensation of the chromatin which ultimately produces the haploid number of chromosomes of the maturation mitoses. These changes will be followed more in detail.

Cytoplasm. The cytoplasm of oogonia (figs. 2 to 6) in fixed material is finely granular, closely packed, evenly distributed, and stains rather deeply. As the oocyte is formed and begins its growth, the cytoplasm may become slightly vacuolated (figs. 7 to 9) with the granules less closely packed and not staining so deeply. As a rule, however, in oocytes which have just started their growth there is little vacuolation of the cytoplasm and these cells differ mainly in a lighter appearance in stained sections. There comes a time after growth is well under way when the cytoplasm begins to assume the alveolar arrangement characteristic of large ova. Usually this vacuolation begins in the cell on the side away from the eccentric nucleus (fig. 10) and the vacuoles commonly possess the central kernel shown in the figure.

The cause of this vacuolation may be the segregation of the less dense substances of the cytoplasm, but the nucleus seems to exercise some control over the process. Figure 9 shows what is sometimes found, viz., a liberation of substances from the nucleus which cause a liquefaction of the cytoplasm about them. This is very evident in figure 11, where deeply staining granules are present in the center of each of the larger vacuoles of the cytoplasm. It is not at all clear whether nuclear substances

are the cause of the type of vacuolation shown in figure 10, since this characteristic group of vacuoles begins its formation at about the place shown, and in these vacuoles deeply staining granules are never found. There is, however, usually an inclusion in the center of these small vacuoles. Certainly there is not the distinct correlation between the liberation of nuclear substances and the vacuolation of the cytoplasm which was found in *Campanularia*. It is worthy of note that there are no yolk spherules present in the cytoplasm of *Aglantha* at any time, while in *Campanularia* yolk spheres were abundant and were produced as the result of the liberation of substances from the nucleus.

As the egg grows it comes to be very markedly alveolar in appearance as the figures in plate 2 show, and the vacuoles are often very large. In these larger vacuoles of later growth stages there are no inclusions, but in growing eggs which show such inclusions there is clearly a rapid liquefaction and absorption going on, hence their absence from mature eggs may merely mean that the dissolving process has been completed and the substances originally present in the granules have been absorbed or else changed to a liquid which fills these spaces.

Nucleus. The nucleus of oogonia is relatively very large, occupying nearly the whole of the cell, displays no reticulum or structural arrangement of any sort, but is filled with a close packed mass of fine granules, and contains a very prominent nucleolus. The structure, staining reactions and general appearance of the nuclear contents are so similar to those of the cytoplasm that only with an immersion lens can a delicate nuclear membrane be defined. The nucleolus appears to contain a great deal of chromatin; perhaps most of that which is in the nucleus is concentrated therein, but achromatic material is present also; the nucleolus, therefore, has a mixed composition. In most sections there is a clear space about the nucleolus, doubtless due to shrinkage. Figure 2 represents a typical oogonium.

In those cells which are clearly oocytes (fig. 7), i.e., in which growth has begun, there is at first little difference in appearance from the oogonia. The nucleolus is usually single, but occa-

sionally there may be more than one (fig. 7). The first indications of change in the nucleus of the growing oocyte are merely the occurrence of rather illy-defined spots and blotches in the nucleoplasm, as though the chromatin of the nucleolus was being distributed over the rest of the nucleus. Figure 7 illustrates these early changes; it also shows that both nucleolus and nucleus have increased in size along with the increase in size of the cell itself. The nucleolus is somewhat lighter in color, as though it were losing some of its chromatin. That such is indeed the case is clearly shown in oocytes slightly older, for the nucleolus becomes noticeably less dense, may show vacuoles, and a distinct nuclear reticulum begins to form during the progress of these changes. In the further progress of these changes the reticulum becomes distinct, develops spots of denser appearance here and there (fig. 8) which become the nodes of the network. The meshes of the net become wider, the chromatin grains coarser and at the same time the nucleolus loses so much of its chromatin as to stain very faintly (fig. 9), and the entire nucleus now has a clear appearance, sharply differentiated from the cytoplasm.

It is about this time that signs are first evident of nuclear material in the cytoplasm. In the early period this is not very abundant but it is clear, as shown in figure 9; such emission of nuclear substances continues and becomes more abundant as the germinal vesicle approaches the time of the maturation mitoses (fig. 12). The substances passed into the cytoplasm first show as sharply defined grains or masses, but they soon disappear, probably by solution. If this material plays any specific part in the metabolism of the cell there is no marked indication of it, except for the formation of vacuoles about the dissolving material. A somewhat similar process was observed in *Campanularia*, but here the result was to produce yolk.

From the stage represented in figure 9 through that of figures 10 and 11 the nuclear reticulum becomes increasingly coarser, the chromatin grains in the reticulum larger and denser, and the nucleolus also larger, but much paler, till it scarcely stains. This is the condition found in oocytes which are well along in their

growth, and in the older gonads all of the larger oocytes have this appearance, with the germinal vesicle large and sharply marked and the chromatin rather uniformly distributed along the reticulum. As the time of the maturation mitoses approaches, the chromatin of the reticulum draws into masses (fig. 12) which gradually condense to form the haploid number of chromosomes, the nuclear membrane disappears, the chromosomes which have assumed their definitive shape are set free (fig. 13), the spindle forms and the chromosomes enter the spindle. Figures 12 to 14 show that there is an excess of chromatin in the nucleus not used for making the chromosomes and this is set free in the cytoplasm and dissolved, along with that which earlier left the nucleus. Before the chromosomes enter the equatorial plate they may show indications of splitting (fig. 13) preparatory to their separation in the anaphase.

In the formation of the polar bodies there is nothing unusual nor especially noteworthy except the very large size of the chromosomes and the remarkable clearness of the various phases of the process. It seems possible that the process of polar body formation goes on slowly, for the different phases were extremely common and were found in hundreds of eggs within the gonads. In the first maturation spindle there are no asters and no centrosomes or attraction spheres, the spindle being rather barrel-shaped. The chromosomes vary in the forms assumed during the anaphase of division, showing rings, dumb-bells, crosses; probably all of these are expressions of differences in the attachment of spindle fibers and in the way the chromosomes are pulled apart. Figures 19 to 24 show some of the phases of division seen in the first maturation mitosis. There is some variation in the time of separation of the chromosomes, which may result in a delayed division of some of the chromosomes (fig. 14).

The polar body is rather large and contains eight chromosomes, which is the number retained in the egg. The chromosomes which remain in the egg draw together into a group (figs. 15, 16, 28, 29) which produces a vacuolation of the cytoplasm, perhaps forming several vesicular vacuoles each enclosing one or more of the chromosomes. There is never the formation of a

membrane about these vesicles, never a fusing of the chromosomes, and no loss of identity of the various chromosomes at any time in the interim between the formation of the first and second polar bodies. While they are still clumped together, and before the second maturation spindle begins to form, there may be a splitting of these chromosomes (figs. 15, 29) in preparation for the next division. Occasionally this splitting for the second maturation division was foreshadowed as early as the anaphase of the first division (fig. 23).

The second maturation spindle forms almost immediately after the completion of the first mitosis, the spindle having the same form as the first and, like it, lacking asters and centrosomes (fig. 17). The second polar body is smaller than the first. So far as observed the first polar body never divides though the chromosomes may start to split longitudinally and then gradually degenerate. The polar bodies themselves degenerate and grow smaller after they have been formed (fig. 18) and finally disappear. The egg nucleus, reformed from the chromosomes left in the egg after polar body formation, is much smaller than the germinal vesicle and of a distinctly different appearance (fig. 18). There is no nucleolus, no coarsely grained, wide meshed reticulum as found in the germinal vesicle (fig. 12), but a vesicular nucleus with the chromatin more or less localized along the nuclear membrane. Possibly a definite reticulum may form later.

All of these changes occur while the egg is still within the ovary. While the second polar body is being formed, or more commonly after this has happened and the egg nucleus has formed, the egg is liberated from the ovary by a rupture of its outer wall. Eggs which have passed through the maturation process have also been found in the cavity of the ovary. They would probably escape from here a little later by a break in the entire wall of the ovary; they are too large to pass into the radial canals, and the latter method of escape is not one which takes place in the hydrozoa, though it is the usual one in the scyphozoa. Fertilization must take place after the eggs have escaped from the ovary and are free in the water, for in none of

the eggs in, or attached to, the ovary was there any sign of fertilization or even of spermatozoa.

Chromosomes. Since the chromosomes in the maturation mitoses are large, it is possible to make some study of their behavior. In referring to the chromosomes of the equatorial plate of the last oögonial mitosis (figs. 5, 6) attention was called to the difficulty of grouping these bodies in pairs, and it was suggested that in synapsis the pairing must be between unlike chromosomes. If this be the case, the two maturation mitoses should demonstrate it, since one of the divisions should separate whole chromosomes while the other would involve splitting of the chromosomes. Figures 19 to 24 are metaphases of the first maturation mitosis and figures 25 to 27 are polar views of the equatorial plates of the same mitosis. In each of the figures 20*a*, 21*a*, 22*a*, at least one chromosome shows the two parts unequal in size. Also figure 23 (which includes only a portion of the spindle) shows that two chromosomes, in dividing, have formed parts which are not quite equal in size. The differences in these cases are not great, greatest in figure 20*a*, but they exist and appear to be characteristic. In the equatorial plates it is difficult to see any difference, but on account of the shape of the chromosomes and the fact that they do not lie in the same plane, slight differences would probably not be apparent.

Figures 15 and 29 represent the group of chromosomes at the inner end of the first maturation spindle and in them a split is already occurring in preparation for the next division. In both these cases there is no doubt of the equivalence of the two parts of the chromosomes. The side view of the metaphase of the second spindle shown in figure 17 also suggests an exact halving of the chromosomes, and polar views of the second maturation mitoses (fig. 34) indicate the same thing. Perhaps figure 23 is the clearest case; here the two chromosomes are just separating in the first division but the second division is foreshadowed in the split in the daughter chromosomes. The parts which are separating are not quite the same, while the line of division in them indicates that the next mitosis will divide them into exactly similar parts. All this, then, is evidence that in the first maturation

tion mitosis the chromosomes which go to the different poles may not be alike in size; there may be a quantitative difference in the daughter chromosome masses.

These facts would also clearly establish another point, viz., which of the two mitoses was a reducing and which an equational division. Since the synaptic mates have not been exactly alike in some of the chromosomes it is certain that the mitosis in which the division of chromosomes produces some unlike parts must mark the separation of whole chromosomes united during synapsis. And likewise the mitosis resulting in an exact halving of chromosomes indicates a splitting of chromosomes and therefore an equational division. From the evidence presented the first division, therefore, is a reduction division and the second is the equational. It might be added that in spite of the apparent absence of a stage which looks like the usual synapsis stage, the behavior of the chromatic bodies in the maturation mitoses gives evidence that a conjugation has occurred.

Another point of some interest may be considered, viz., Are the chromosomes of different generations related? This does not involve a morphological continuity, for at the present time such a thing is admitted not to be possible. But do chromosomes of one generation arise from particular chromosomes of a previous generation? If we compare the chromosomes of oogonial mitoses (figs. 5, 6) with the chromosomes of the two maturation mitoses of the oocytes (figures of plate 3) it will appear that there is not a great similarity in form and size of the chromosomes of the different generations. The figures showing the chromosomes in the equatorial plate stages are most favorable for this comparison. In the oocyte mitoses there are always four large and four small chromosomes. In the oogonia it is not so certain that there are eight smaller and eight larger chromosomes, though there are some which are large and others which are small. The differences in size in the chromosomes of one spindle or of spindles of different cells do not seem to be constant, for an examination of the various figures which show chromosomes will at once make it clear that the chromosomes are not the same relative sizes in every case. While differences are also evident in the shapes of chromosomes the use of this as a criterion of com-

parison is not so satisfactory since the point of view of the chromosomes would make a greater difference in apparent shape than in size. However, the comparison of shape may be made in the same manner as that of size and the results will be the same.

Because of the apparent lack of constancy in the size and shape of chromosomes, it hardly seems possible that there is a reappearance of the same chromosome in succeeding generations. Such evidence as we get does not warrant the conclusion that the chromosomes are the same in size and shape in every cell, generation after generation. The evidence does not, perhaps, permit us to conclude that the chromosomes of succeeding generations are distinctly different in size and shape, but it seems best to remain somewhat skeptical with regard to the genetic relationship of chromosomes till the evidence demonstrates it beyond question.

It may be stated that although the material here used, the medusa *Aequorea victoria*, is far more satisfactory for a study of chromosomal behavior than any other coelenterate known to me, it may still not be so favorable as material obtained in other phyla.

And yet it is certain that this material is far more favorable than that of some other animals. This would be obvious to one who has seen this material and that from other sources. In a cytology course given to students I have used the oogenesis and spermatogenesis of insects, crustacea and vertebrates as material for study; from a knowledge of the character of the chromosomes of these forms I have no hesitation in saying that the chromosomes of *Aequorea* are as satisfactory for a study of chromosome behavior as most of the groups mentioned.

CONCLUSIONS

The germ cells of *Aequorea* are formed by a proliferation of the ectoderm cells of the sub-umbrella in the aboral region of the bell. The division of these cells continues for a time, always by mitosis, until a large number are present in the gonad when the division period ends, at about the same time in all the gonads of the individual. The nucleus of an oogonium is large and the nuclear contents so like the cytoplasm in appearance as to be

distinguished only with difficulty. Within the nucleus is a large, deeply staining nucleolus.

In the last oogonial division there are 16 chromosomes differing in shape and size, but not paired either in arrangement or in form and size. If conjugation of these occurs, as maturation mitoses seem to indicate, the synaptic mates are unlike in some cases. No contraction (synizesis) or conjugation (synapsis) stages could be found, though the material was favorable in character and in age.

In growing oocytes the cytoplasm becomes vacuolated, finally assuming an alveolar appearance; during growth there is a passage of chromatin from the nucleus into the cytoplasm. This extra-nuclear chromatin produces some vacuoles in the cytoplasm as it gradually disappears. The nucleus comes to have a coarse reticulum of deeply staining chromatin and, as this forms, the nucleolus becomes very faintly staining. Eight chromosomes (the haploid number) are formed and pass into the maturation spindle, the latter being without asters or centrosomes. Some of the chromosomes in the first maturation mitosis may lag behind the others in division. The first maturation mitosis is reductional, the second equational. The two polar bodies gradually become smaller and finally disappear. The chromosomes left in the egg form the egg nucleus in which the chromatin is localized against the nuclear membrane. After maturation the egg is liberated from the gonad and fertilization takes place in the water.

A study of the chromosomes shows that: their conjugation in synapsis may take place between unlike members; the chromosomes of the oogonial divisions are not the same in form and size as the chromosomes of the maturation divisions; chromosomes of the maturation divisions are not exactly alike in different eggs; there is no evidence that the chromosomes of one generation are descended from particular chromosomes of a previous generation, the contrary seems to agree more nearly with the evidence.

The chromosomes of *Aglantha* are as favorable for a study of chromosome behavior as many, and more favorable than some, of the other phyla which have furnished material for such studies.

IV. HYBOCODON PROLIFER

TWENTY FIGURES (THREE PLATES)

STRUCTURE AND HABITS

Hybocodon is one of the Tubularian hydroids with the general structural characteristics of that group, but has especial interest in the fact that the medusae which are produced give rise to other medusae by budding. Thus, not only the polyp produces buds asexually but the medusae do the same thing. The hydroid is recorded as having been found but rarely on our Atlantic coast, but it must be fairly abundant in places about Cape Cod and elsewhere since the medusae are sometimes very abundant. Agassiz ('62) found it in very quiet and protected waters about Nahant and believed its normal habitat was in deep water in places protected from waves and strong currents. Such a habitat would explain why the hydroid is rarely captured, for such a position would render it difficult to obtain by dredging.

The hydroid (fig. 36) is a solitary form, or at most a colony consists of a few unbranched stems connected by branching stolons. The hydranth at the end of the stem is rather large, has a single row of basal tentacles and a double row about the mouth. Figure 36 is drawn from a preserved specimen and is satisfactory in showing the general form; a better representation is the figure of Agassiz ('62, vol. 4, Plate 25) which is drawn from life. The medusae arise by budding from the sides of the hydranth between the basal and apical series of tentacles, and are produced in large numbers from a single polyp. The medusae have four radial canals with swollen bulbs at their bases, but only a single tentacle which extends from one of the bulbs. The bulb at the end of this tentacle is very much enlarged, and this, with the single tentacle extending from it, gives the medusa a very unsymmetrical form. Figures 37 and 38 are drawings

made from preserved individuals, both figures show the general character of the medusae, and its habit of budding other medusae.

The medusae produced by budding from the base of the single tentacle are liberated to become free swimming and independent individuals. A good many of these secondary individuals may be formed from the primary medusa and as they in turn produce new medusae by budding there is a great lengthening of the life cycle of the animal and a tremendous extension of its range. While there are commonly a good many buds present in various stages of development on any one individual, it may be that others will show only a single bud or be without buds. The developing medusae may begin to produce small buds even before they are themselves liberated, and it is possible to find several generations present at the same time. On the other hand the budding process may begin only after liberation. The budding continues for a long season since medusae captured at almost any time in spring, summer, or autumn possess the buds.

In addition to this asexual method of reproduction in the medusae there is the usual sexual method. Each medusa is a sexual individual and produces either eggs or sperms on the sides of the manubrium (figs. 38, 39). The eggs are not discharged from the gonad but are fertilized in place, and development of the eggs proceeds till a young polyp or actinula is formed. The actinula is freed from the parent medusa and starts the new hydroid or polyp generation. Figure 39 shows a section of such an actinula on one side of the stomach wall of the medusa and a part of the ovary on the other side. This fact of sexual and asexual reproduction occurring in the medusa has been known for a long time; the same condition exists in a few other medusae also, though asexual reproduction is not common in medusae. Furthermore it has been shown that both methods of reproduction may take place at the same time. C. W. Hargitt ('02, '04) and Perkins ('04) have both noted the occurrence of these two methods at the same time and Mayer ('07) calls attention to it. A large number of specimens examined by the author showed no correlation between the time of formation of the medusa buds and the formation of the germ

cells. For, medusae were found with the sexual products only, others possessed buds and no sexual products, and still others showed buds in abundance and sexual products forming or actinulae developed. Perhaps nearly one half of the specimens had both buds and sexual products present. The sexual and asexual reproductive processes of the medusae may thus occur simultaneously, or the buds may develop and be liberated before the sexual products form. Since all these conditions are found in medusae taken at the same time and from the same place, and since there is no correlation which can be observed between the size of the medusae and the presence of buds, it is probable that budding is not a rhythmic or periodic process. Buds have been found on specimens in the autumn (C. W. Hargitt '04), and Agassiz ('62) found two generations of buds present before the medusa was liberated from the polyp. Probably, therefore, budding is an expression of healthy activity, vigor, and plentiful food much as it is in hydra, and it doubtless occurs during the entire life of the medusa. The sexual organs develop at rather definite seasons, March to May being the season recorded by C. W. Hargitt ('04); but this season is not sharply limited, for I have found specimens later in the summer which possessed gonads.

From the description of the structure and reproductive habits of these medusae it is evident that the material is almost ideal for an investigation of the formation of the germ cells and their possible migration from the older medusae to the budding secondary and tertiary ones. In other words, if there is a continuity of the germ plasma it should be possible to demonstrate it in this form by finding germ cells migrating into the young medusae which are developing asexually. The cells and tissues of the medusae are greatly specialized and differentiated, far beyond the amount of specialization in polyps. The cells of the bell, canals and tentacle bulbs are entirely unlike any germ cells in appearance; any of the latter cells should easily be recognized if present. The ectoderm cells of the stomach of the medusa are more like germ cells in appearance than any of the other tissues, but even if germ cells could not be distin-

guished here the only way for such cells to get to the budding medusae would be by a migration along the radial canals or through the jelly of the bell. They could not escape notice in these places since the cells of the canals and tentacles are very unlike germ cells.

The study of *Hybocodon* is directed mainly to an investigation of this continuity of the germ plasma, and no attempt is made at a complete study of the cycle of the germ cells, but where any facts are obtained bearing on conditions found in other forms, they will be set forth.

MATERIAL

The material on which this study is based was obtained by several persons, in different seasons, and at different places. By towing a net through the waters of the harbor at Woods Hole, in Vineyard Sound and vicinity some material was secured. However, the greater part was gathered by the author while on a dredging trip with the U. S. Fisheries Steamer *Fish Hawk*. The vessel being anchored, during a fog, in the main channel near Crab Ledge and the Stone Horse Shoals, east of Chatham, the tow nets were lowered into the strong tide current which was running and great numbers of the medusae were collected both from the surface and from a depth of several fathoms.

The technic of fixation included formalin, fluids containing mercuric salts, picric acid, osmic acid, and the like. The formalin fixation was excellent for general morphology but was not very favorable for cytological study. The mixtures were all fairly satisfactory except for a shrinkage of the jelly of the bell which occurred in all of them; in Bouin's fluid there was less shrinkage, and mercuric chloride mixtures also gave very good results. Sections have been made longitudinally and also transversely through the entire medusa, and the study has included careful examination of all sections from the aboral end of the bell to the tentacles, in order to guard against overlooking migrating cells.

GERM CELLS IN PRIMARY MEDUSAE

The germ cells located in the ectoderm of the stomach wall produce a gonad in the form of a thickened ring about that organ (fig. 38). It is not certain that the germ cells take their origin from the ectoderm (critical early stages were missing) but the appearance of the gonad and other tissues of the stomach suggests such an origin. It may be possible that germ cells are present in the wall of the hydroid polyp from which the primary medusae arise, but since the hydroids are so rarely, if ever, seen such a determination can not soon be made. I have seen a single specimen of the hydroid which had been preserved for many years in alcohol and was of no service for cytological study, even if it were not more valuable as a part of a collection of hydroids. It hardly seems probable that the germ cells are present in the polyp since sections of medusae show many individuals in which no such cells are recognizable. The lack of germ cells suggests either an old individual which has shed all its sexual products, or an immature individual in which germ cells are not yet differentiated. Size should be something of a criterion in determining which alternative to accept, and since some of the medusae without recognizable germ cells are smaller than the average we may conclude that these, at any rate, are immature.

When germ cells are present, even if only a few in number, they are easily recognized by their position, size, shape, compact, deeply staining cytoplasm and usually by a large nucleus. On the right side of the stomach wall in figure 39 a portion of the ovary is represented in the swollen mass which is composed of a group of small oocytes. In no case has the gonad been observed to extend further aborally than here shown; there is always a space between the top of the gonad and the place where the sub-umbrella meets the stomach. The same figure (39) shows an actinula or larval polyp in position on the left side of the stomach. This has developed in this position and is about ready to be liberated. Attention is also directed to the swollen base and a portion of the tentacle at the lower right corner of the figure; it is from this bulb that secondary medusae are produced by budding.

ORIGIN OF GERM CELLS IN THE SECONDARY MEDUSAE

The germ cells of the primary medusae are not our chief concern, except for their form and their position relative to the place of formation of secondary medusae. According to the theory of the continuity of the germ plasma all germ cells are genetically related through a direct line of germ cells. There can be no new formation of germ cells, no transformation of body cells into germ cells, but only by a division of a germ cell may another be produced. In Coelenterates in which the sexual individuals may secondarily produce other sexual individuals by asexual means, there should be, on the theory, a migration of some cells from the gonad of the primary individual to the budding secondary individuals.

Hybocodon is admirably adapted to test such a thesis. Figure 39, while a somewhat diagrammatic representation of a longitudinal section of a medusa, is made by carefully combining two or three sections, each of which was drawn with a camera lucida. The outlines of the bell, wrinkled in the sections, are made smooth but no other modification has been made; the form, the size, the relation of parts are absolutely as observed in the sections. The gonad is on the wall of the stomach, and the swollen base of the tentacle, the only point from which medusae bud, is at the lower corner of the medusa at the right of the figure. Germ cells to pass from the gonad to the tentacle base must proceed aborally to the point where the stomach meets the bell, and must then migrate the entire distance through the bell to the tentacle.

Evidence on the migration of germ cells. In the bell migrating cells might go through the jelly, or along the ectoderm of the sub-umbrella, or in some way get into the radial canal and proceed to the tentacle, there to pass into the developing bud. The jelly of the bell is non-cellular and any cell migrating through it would at once be observed; since no cells were ever observed in this jelly the latter is plainly not a path of migration. The ectoderm of the sub-umbrella is a delicate sheet of cells in a single layer so thin as to be difficult of demonstration except with

very high powers. A germ cell in this layer would be almost as obvious as in the non-cellular jelly; no evidence of such cells was found. The radial canals, then, remain as the only probable path along which cells could pass from the gonad to the tentacle. The shortest path would be along the radial canal which leads to the single tentacle from which the secondary medusae develop; if any other canal were followed there would necessarily be a further migration through the circular canal to the tentacle. In order to canvass the situation thoroughly all the canals were examined carefully throughout their length.

Figure 40 is a drawing showing the typical structure of the terminal portion of the radial canals and the enlargements at their distal ends. The figure represents the canal and bulb at the base of the single tentacle, but the same structure is typical of all the canals. The entoderm cells are arranged in a single layer in both the canal and the tentacle bulb, their nuclei are rather small and the cytoplasm is much vacuolated. Figure 41 is a much enlarged and carefully drawn portion of the bulb at the end of a radial canal; the very large size of the cells, their vacuolated cytoplasm more or less filled with large spherules, and the characteristic nuclei, are well represented. Figure 42 is a transverse section of one of the radial canals from the aboral portion of the medusa; the ectoderm layer of the medusa is not shaded but the jelly of the bell and the entoderm cells of the radial canal are shown as nearly like the sections as possible. These entoderm cells, more clearly than those in figure 40, show the character of cell and nucleus, and the structure shown in the figure is typical of the radial canals of all the medusae examined.

Over fifty medusae were carefully examined through every section of each canal and the conditions found were practically identical with those shown in the figures. There is some variation in the number of cells or nuclei present in any one section, but never was there more than a single layer of cells, never was there any cell crowded back of these canal cells against the supporting layer or jelly, never was there a cell whose cytoplasm was compact, finely granular and deeply staining as germ

cells usually are. Consequently one may say that in the many medusae examined there was no evidence of a migration through the entoderm tissue of the radial canals, and no evidence of interstitial cells or primordial germ cells in the tissues of the canals or tentacle bulbs.

The only possibility remaining is whether germ cells may pass through the cavity of the canals to the tentacle and thus to the budding medusae. Such a course is improbable since a passage entirely through the supporting layer and entoderm would be necessary before the germ cells could enter the gastro-vascular cavity. It is also probable that germ cells in the gastro-vascular cavity would be subject to digestive action of enzymes and could hardly escape some disintegration as a result. Of all the medusae examined the only cases found where solid matter was present in the canals are illustrated in figures 43 and 44. The rarity of this condition is probably indicative of its unusualness, but these inclusions will bear careful scrutiny. Figure 43 is a longitudinal section of the aboral portion of a radial canal near the point where it joins the large marginal bulb. The substance within the canal extended over several sections but there was not, in either mass, any sign of a nucleus; there were a few deeply staining spots which might have been of nuclear origin, but these were small and scattered. The cytoplasm was much vacuolated and filled with large and small spherules of different composition or density, and the edges of the masses were irregular and broken. Such masses resemble, somewhat, the tips of cells like those shown in figure 41, and do not look like germ cells. But whether they might be considered as germ cells is of no significance since their condition shows clearly that a disintegration is taking place. They could not, therefore, be active and functional cells.

Figure 44 gives us a different picture. This is a section of a radial canal near its union with the stomach. The entoderm cells of the canal are like those in figures 42 and 43 and there is no difference in the ectoderm and jelly. The cell which is within the canal does resemble a germ cell in some ways in that there is a deeply staining, finely granular and compact proto-

plasm; but the dense, spherical body resembles a nucleolus rather than a nucleus. If the mass represents a nucleus and a nucleolus only, it is of no significance to us, for it must be a portion of a disintegrated cell. If it is a cell with a small homogeneous nucleus it is probably not a germ cell, for such is not the characteristic form and proportion of a germ cell. There are some signs of cytolysis evident which leads one to consider this as a degenerating cell. But if we assume that it is a germ cell we find it is as large, almost exactly the same in size, indeed, as one of the young oocytes of the stomach wall. No cells of medusa buds are more than one-quarter the size of this cell which is within the cavity of the canal (compare figure 44 with figures 46 to 50), nor are any cells of the medusa buds just like this in appearance. From these facts I incline to the view that this inclusion within the radial canal is a cell, a germ cell if you please, but an abnormal, partly disintegrated cell accidentally in this position.

There is, then, no evidence at all of a migration of germ cells from the gonad of the main medusa to the budding secondary medusae. This conclusion is based on careful investigation of over fifty individuals examined at every possible path of migration.

Evidence on the presence of germ cells in the budding zone. Another possible way in which to account for the presence of germ cells in the secondary medusae is to assume that when the primary medusae were first forming, some germ cells were located in the tentacle where they would later be needed to produce the germ cells of the buds. This would be more plausible if all future generations of medusae were present on the main medusa before it left the mother polyp: a grouping of germ cells might occur in each bud rudiment ready to produce the gonads. But as there is an extensive formation of buds from free medusae, buds which begin to develop only after the main medusa is liberated, germ cells should be present, if at all, in the budding zone (i.e., the tentacle bulb) awaiting the opportunity to pass into the buds as they start their development.

On the premise we have made this must be so, for it has been demonstrated that there is no migration of germ cells from the gonad of the main medusa to its buds. A study of sections of the bell, the canals, and the tentacle bulbs has also made it clear that no germ cells can be recognized in the entoderm of the canals or tentacles, nor in the jelly or sub-umbrellar ectoderm of the bell. It remains to be determined whether there are germ cells present in the ectoderm of the tentacle bulb. It is also desirable to trace the course of development of the medusa buds and see when and where the germ cells first appear.

Figures 40 and 41 show the characteristic appearance of the cells of the tentacle base, figure 41 showing especially well the large, much vacuolated entoderm cells containing granular inclusions, and the more or less vacuolated ectoderm cells. The entoderm cells are distinctly of the type of those in the gastro-vascular organs. Careful attention was paid to the bases of these cells, but there were no small cells interspersed, no interstitial cells, nothing in any way resembling a germ cell. The ectoderm cells likewise are in a single layer, there are no interstitial cells here, and neither the cytoplasm nor the nuclei of these cells reminds us of germ cells. It seems that there are no cells in the tentacle bulb which may be recognized as germ cells, indeed none of these cells has the slightest resemblance to germ cells.

A minute and careful search of all parts of the main medusa has shown that there is no migration of germ cells from this to the developing buds; there are no cells in the canals or the marginal bulbs at the ends of the canals which contain germ cells; the large tentacle bulb from which the medusa buds arise possess no cells either in ectoderm or entoderm which could be called germ cells or mistaken for them; even the budding zone of the bulb shows no indications of germ cells being present. It seems fair to conclude, from all this, that germ cells are not present as such anywhere in the medusa except in the gonads. The gonads which arise in the secondary medusae must then develop quite independently of any germ cells in the main medusae. A study of the development of the medusa buds is, therefore, of importance in determining the origin of these cells.

DEVELOPMENT OF MEDUSA BUDS

The first indication of the new bud is an outgrowth from the tentacle base, and this outgrowth includes both the ectoderm and the entoderm of the tentacle. Figure 45 shows a small and a larger evagination, both of which are to produce medusae. In the drawings of such a scale the character of the cytoplasm is not represented, nor is the nucleoplasm indicated; this is not a serious omission since it may be said that all the cells of these outgrowths are practically identical in appearance and character of their contents. The cytoplasm is a compact, non-vacuolated, rather deeply staining substance, the nuclei are all large and filled uniformly with granules and contain a prominent nucleolus. There is some difference in the shape and size of these cells but these are the only distinctions to be observed in the evaginations.

Figure 46 is a slightly later stage in the formation of the medusa bud, the ectoderm growing inward and pushing the entoderm back before it. Figure 47 is a bud of about the same stage of development, or slightly earlier, and magnified somewhat more, in which the ectoderm cells are increasing in numbers but have not yet grown so far inward. Some of the entoderm cells are assuming a columnar form while the ectoderm cells remain polygonal. Figure 48 is an older bud in which the proliferating cells of the ectoderm are growing inward on either side in several sheets and pushing the entoderm cells still further back. The sheets of ectodermal cells growing inward on the two sides have already split into two layers and these are to form the ectodermal lining of the sub-umbrella and the ectodermal covering of the stomach in the adult medusae. The two strands of entoderm which extend furthest toward the outer margin of the bud are beginning to form two of the radial canals. In nearly all of these stages it will be seen that the entoderm cells are the first to change their form and assume something of the columnar shape characteristic of such cells in mature medusae. Likewise in all these stages the ectoderm cells remain polygonal in form.

Where in the stages described so far are the germ cells developing? It is impossible to call any of these cells, whether of ectoderm or of entoderm, primordial germ cells, for the reason that they are all so similar in structure and appearance, and most of them so nearly alike in form and size as to render any distinction impossible. All the cells of these buds have the appearance of rapidly growing, undifferentiated cells, that is, all are like embryonic cells. The same appearance is found in the vegetatively developing buds of hydroids, and indeed the growing zones of many animals have the same general appearance as those in these developing medusa buds. In these buds, even in stages much older than that shown in figure 48, some of the entoderm cells are so like the ectoderm cells as to make a distinction difficult or impossible. The boundaries of the tissues are clearly represented in figure 48 for the sake of distinctness, while in reality it is only with oil immersion lenses that the boundary lines can be certainly determined. In figure 47 it is uncertain whether the group of cells at the lower part of the bud represent ectoderm cells wholly or whether some of them belong to the entoderm; there is a boundary on both sides of the group of cells, and the shape, size, and appearance of the cells does not sharply differentiate them.

In a stage considerably later than those described, when the bud has assumed something of the outline of the medusa, and when the stomach is well formed and the canals are laid down in a general way, a section through the stomach wall has the appearance of figure 49. The entoderm cells (to the right in the figure) are beginning to show the vacuolations and granular inclusions characteristic of gastrovascular tissues. Gland cells are also forming. These cells are assuming their definitive form, and the presence of gland cells shows they are ready to assume their definitive functions, if they are not already functional. The outer layer of the stomach, the ectoderm, in which gonads will eventually develop, is still composed of the embryonic cells. Over the greater portion of the stomach the ectoderm is a single layer thick, but about the outer end there is a mass of cells; this end of the stomach is still increasing in size.

The next older stage to be considered is one in which the bud has increased in size and has the general shape of a tiny medusa. The various parts are all present as rudiments except that the jelly has not yet begun to form, but none of the organs or parts is more than rudimentary. A section through the stomach wall of such a bud is shown in figure 50. The entoderm cells are as characteristically gastrovascular cells as are those in a similar position in an adult medusa, but none of the other tissues of the medusae are characteristic. The ectoderm of the stomach (right in figure 50) is composed of a single layer except at one point where a mass of cells extends part way around the stomach. This group of cells was near the oral end of the stomach but not at the tip; these cells may not be simply the embryonic cells of the growing medusa, though there is practically no difference between them and such embryonic cells. More than any other cells so far found and described, these cells resemble primordial germ cells, though I fancy this resemblance is assisted by our knowledge that this is the place where the gonad is eventually to form. If we take it for granted that these are primordial germ cells let us note at what a relatively late stage they develop. The developing bud is of considerable size, all the organs and parts of the medusa have been laid down as rudiments and some parts, as the entoderm of stomach, have taken on their definitive form and are functional. The gonad does not even exist as a rudiment till relatively late in the development after all these other parts are started. Furthermore the gonad is developed, not from any localized group of cells but from the general mass of more or less embryonic tissue which has earlier been the source from which all other tissues and organs have taken their start.

Since it has been shown that there are no recognizable germ cells in the tentacle bulb from which the medusa buds arise it must be that the buds have come from the ordinary cells of this region. The cells of the tissues of this budding region (figs. 40, 41) show beyond question that the cells here are differentiated. By the very side of the developing bud the ectoderm may be forming nematocysts (at the right in figure 45) and the

entoderm cells bordering and extending into the developing bud are distinctly the gastrovascular tissue of the medusa (fig. 40, 41, 45). At a similar point on the tentacle bulb of individuals which are not producing medusa buds there is nothing to distinguish the tissues from those of any other part of the tentacle. In other words the medusa buds take their origin from a region of differentiated and specialized cells.

But the cells of even the early outgrowths are distinctly of an indifferent or embryonic character; the differentiated cells must, therefore, undergo a modification, a regressive change, at the time and in the place where the bud starts. Tissue cells in this medusa must, therefore, have the capacity to give rise to embryonic tissues capable of again differentiating into tissues specialized morphologically and physiologically. In short they may produce new individuals with their varied cells, including germ cells, for the germinal tissue arises from the same indifferent tissue of the bud. Before the body cells may thus produce a new individual they must undergo a dedifferentiation.

Such a capacity of tissue cells may be interpreted by some simply as an indication of their possession of that peculiar substance called germ plasm. There is no apparent reason why such an explanation may not be given, though it obviously broadens the significance of the term far beyond what was originally, and is still usually, given to it. It must be clear, however, that if this is the significance of the term 'germ plasm,' or rather if the term may be so broadened in its scope as to include this concept, the term itself has no meaning and had better be discarded. For such a capacity of cells as that described merely means that tissue cells may produce a new generation either by budding, or by first forming germ cells which in the usual manner develop into a new individual. This is precisely the conclusion to which the study of this medusa and of other Coelenterates has led me.

For those who desire to consider the indifferent cells of the developing medusae as potential germ cells, since they are rather embryonic in character, there is a further complication. In the tentacle bulb, in the absence of developing medusae, no such

embryonic cells can be found. The differentiated cells must undergo a regressive change and themselves become the embryonic cells both in appearance and potency. If all the cells which are embryonic may be considered as germ cells the cells from which they arise are entitled to the same name; but the original cells are differentiated body cells, and this means body cells may give rise to germ cells. This is a direct contradiction of the germ plasm theory.

GROWTH OF OOCYTES

In mature medusae the germ cells extend in a swollen mass about the middle of the stomach, forming a well marked gonad. The cells within this gonad are numerous, closely packed together, and are alike in size and structure in the early stages. In the ovary only a few of these cells grow to produce mature egg cells, the ones which get the start are the ones which grow, the others remain unchanged and serve as food for the growing cells. The oocytes which fill the ovary in a gonad in which no growth has occurred have the structure and appearance indicated in figure 51. In the ovary from which these cells were taken there was no large ovum and only a single oocyte had begun to grow (fig. 52). The oocytes have a deeply staining compact cytoplasm with a fairly large nucleus, faintly staining, and a large deeply staining nucleolus. The latter with iron-hematoxylin stains an intense black; with hematoxylin and eosin the acid stain is the one which stains the nucleolus, though the hematoxylin may stain the nucleolus slightly along with the eosin. There may be a few vacuoles in the nucleolus at this time but as a rule it appears homogeneous. The rest of the nucleus stains very faintly in iron-hematoxylin and a little more with Delafield's or Ehrlich's hematoxylin. There is a fairly even distribution of this nucleoplasm, with a very faint hint of a delicate reticulum in a few cases.

In oocytes of this age, that is before growth, there is often an accumulation of more deeply staining material in the nucleus arranged in a ring about the nucleolus (fig. 51). In every oocyte there is a more or less rich accumulation of deeply staining

granules in the cytoplasm close to the nuclear membrane. Some of these granules are slightly removed from contact with the nuclear membrane and a few may even be close to the outer border of the cell (fig. 51). This is clearly an indication of a migration away from the nucleus, and is also indicative of the origin of this material within the nucleus.

When the oocyte begins to grow these granules cease to form and such as are present migrate further into the cytoplasm (fig. 52) and there finally disappear. While still visible within the cytoplasm they have a tendency to cause a vacuolation of the cytoplasm about themselves. This marks the same sort of chromidia formation as has been observed in other Coelenterates, and other animals. In this form it is of slight duration but it may play a definite part in the life of the cell in spite of the rapidity with which such a stage passes. It is quite possible that the fact of a different sort of nutrition in *Hybocodon* (absorption of the sister oocytes) than in such forms as *Clava* and *Campanularia* (absorption of liquid food material from the gastrovascular cavities) has something to do with the difference in the sort and amount of chromidia formation.

In the beginning of growth increase is largely within the cytoplasm, the nuclei not growing much; the nucleus in the growing cell of figure 52 is only slightly larger than those in the oocytes which are not growing (fig. 51), though the cytoplasm has increased twofold or more. The nuclear contents in the growing oocyte are arranged in a reticulum, but there has been no change in the staining reaction and so probably no chemical change. It is only after the oocyte has increased to a considerable size that the absorption of the smaller oocytes takes place to a very large extent; in a few cases the presence of two germinal vesicles within one large oocyte shows that there has been a fusion of oocytes. The oocyte sends out pseudopodial extensions of considerable length and in considerable numbers, and these prolongations extend between the smaller cells feeding upon them either by absorption or engulfment. This is a common method in *hydra* and some other hydroids. The nucleus of the absorbed cells is more difficult to dispose of than the cyto-

plasm and the growing cell may be more or less filled with these disintegrating nuclei and nucleoli (fig. 53). Muller ('08) has described this in *Hybocodon* and it was recognized by Kleinenberg ('72) in hydra and by all who have since worked on hydra; Wager ('09) has made a careful study of these nuclei, or 'pseudo-cells,' in hydra. Many other authors have described such conditions as found in various hydroids.

In an egg in which the feeding is still progressing and whose pseudopodia are still extended (fig. 53) the cytoplasm has become filled with vacuoles and presents a distinctly alveolar appearance. The dissolving nuclei of the absorbed oocytes usually lie within some of the vacuoles and bodies of a different sort occupy other vacuoles. In the particular egg figured the germinal vesicle was without a nucleolus, though usually at this stage of growth a nucleolus is present. The nuclear reticulum is somewhat coarser than formerly but stains no more deeply indeed at this time it shows a tendency to select a cytoplasmic rather than a nuclear dye. In place of a nucleolus the egg described had eight to ten small deeply staining bodies which may be fragments of the nucleolus, or perhaps chromatin bodies.

The extent to which a growing egg forms pseudopodia is well shown in figure 55. This is a reconstruction of the stomach and growing eggs of one individual studied. There were a great many small oocytes present all about these eggs, filling the spaces and making up the rounded gonad; these have been omitted from the reconstruction. There were two, perhaps three, large growing eggs in this gonad, it is uncertain whether the lower mass on the right of the figure is a distinct and independent egg or a portion of the one above it. The egg on the left of the stomach has abundant and uniformly distributed pseudopodia, the large one on the right has but few outgrowths; probably both eggs are approaching maturity.

When the egg has reached its full growth the pseudopodia are withdrawn, the cytoplasm rounds out and a globular ovum is produced. The germinal vesicle and a portion of the cytoplasm of such an egg is shown in figure 54. The cytoplasm is much vacuolated, no sign of the absorbed oocytes remains, but

a group of granules is present close to the germinal vesicle. These have the same appearance as the granules which were present at the very beginning of growth (fig. 51), but their origin is not very certain. Within the germinal vesicle the reticulum is still faintly staining and similar to its earlier condition. There are, however, chromatin strands in a group about the remains of the nucleolus. Since this egg has reached its full growth the chromatin strands represent the beginning of the formation of chromosomes.

Further stages in maturation have not been studied because the material is lacking in some of the essential phases. The actual formation of the polar bodies and the fertilization by the spermatozoon have not been observed in any of the material which I have collected. This is somewhat strange since the material is abundant and shows other phases in considerable number; it may be possible that there is a periodicity in the formation of polar bodies and the material was not collected at the correct time. Also since but two or three eggs ever develop in a single ovary, the number of such eggs at command for study is not very great, even in an abundant supply of medusae; the number which might be expected to show maturation stages would be even fewer. Muller ('08) in his paper on *Hybocodon* also was without this stage, and probably the maturation and fertilization of *Hybocodon* have never been observed.

The egg remains in its place in the gonad after growth and maturation are completed and the fertilization is accomplished in the gonad. The egg then enters upon its cleavage, still within the gonad, and finally produces a larval polyp with tentacles, the actinula. A section of an actinula in position and about ready to be set free is shown in figure 39.

CONCLUSIONS

The medusae of *Hybocodon* give rise to other generations by budding secondary medusae from the base of the tentacle. The medusae are sexual individuals and produce either eggs or sperm. Sexual products are produced more or less periodically, while buds arise at any time; the sexual and asexual reproductive processes often occur simultaneously.

If germ cells originate only from other germ cells, an animal whose sexual individuals produce other sexual individuals by budding might be expected to show a migration of germ cells from the primary to the secondary individuals. If a migration does not occur primordial germ cells must be present in the budding zone, to satisfy the hypothesis mentioned (the germ plasm theory).

An examination of every possible path of migration in *Hybocodon* shows that such a migration does not occur. Careful search of all the tissues of the medusae shows no trace of germ cells, nor of cells in any way resembling germ cells, except in the gonads. The bell and its tissues, the canals, the tentacle and its base (the budding zone) all contain differentiated body cells, but no germ cells. Germ cells in the secondary medusae must, therefore, arise quite independently of any germ cells of the primary medusae.

In developing buds the various tissues and organs of the medusae are all present, at least as rudiments, before there is any evidence of germ cells. The gonad is the last of the organs to develop and no germ cells are present except in the gonad. The medusa buds arise from differentiated cells of the tentacle bulb, but before initiating the new growth they undergo a regressive change and become indifferent or embryonic in appearance and potency. The indifferent cells so formed eventually undergo a re-differentiation and produce the various tissues and organs, including the germ cells. Since the germ cells of developing medusae have thus come from differentiated body cells it is clear that there is no distinct germ plasm in *Hybocodon*.

In adult medusae only a few of the many oocytes of the gonad grow and form ova, the other oogonia and oocytes being absorbed by the growing eggs as food. The germinal vesicle of the growing egg remains very small and the nuclear reticulum appears almost achromatic till the chromosomes begin to form. An emission of chromatin from the nucleus takes place at the beginning of growth.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Aglantha digitalis

1 Portion of young ovary; shows entodermal epithelium of cavity of gonad, oogonia and oocytes, two growing oocytes. $\times 615$.

2 Oogonium. $\times 2000$.

3 and 4 Oogonia from border of oocyte zone; probably in prophases of division; fourteen loops present in figure 4. $\times 2000$.

5 and 6 Oogonia in last division; polar view of metaphase stage of division. The sixteen chromosomes represent the diploid number. $\times 2000$.

7 Oocyte after growth has started. $\times 2000$.

8 Oocyte after considerable growth; nucleoplasm assuming a reticular condition. $\times 2000$.

9 Growing oocyte showing chromatin bodies in the cytoplasm; nucleolus non-chromatic. $\times 720$.

10 Growing oocyte, nuclear reticulum coarse and deeply staining; vacuolation of cytoplasm beginning. $\times 720$.

11 Growing oocyte; extra-nuclear chromatin causing vacuolation of cytoplasm. $\times 720$.

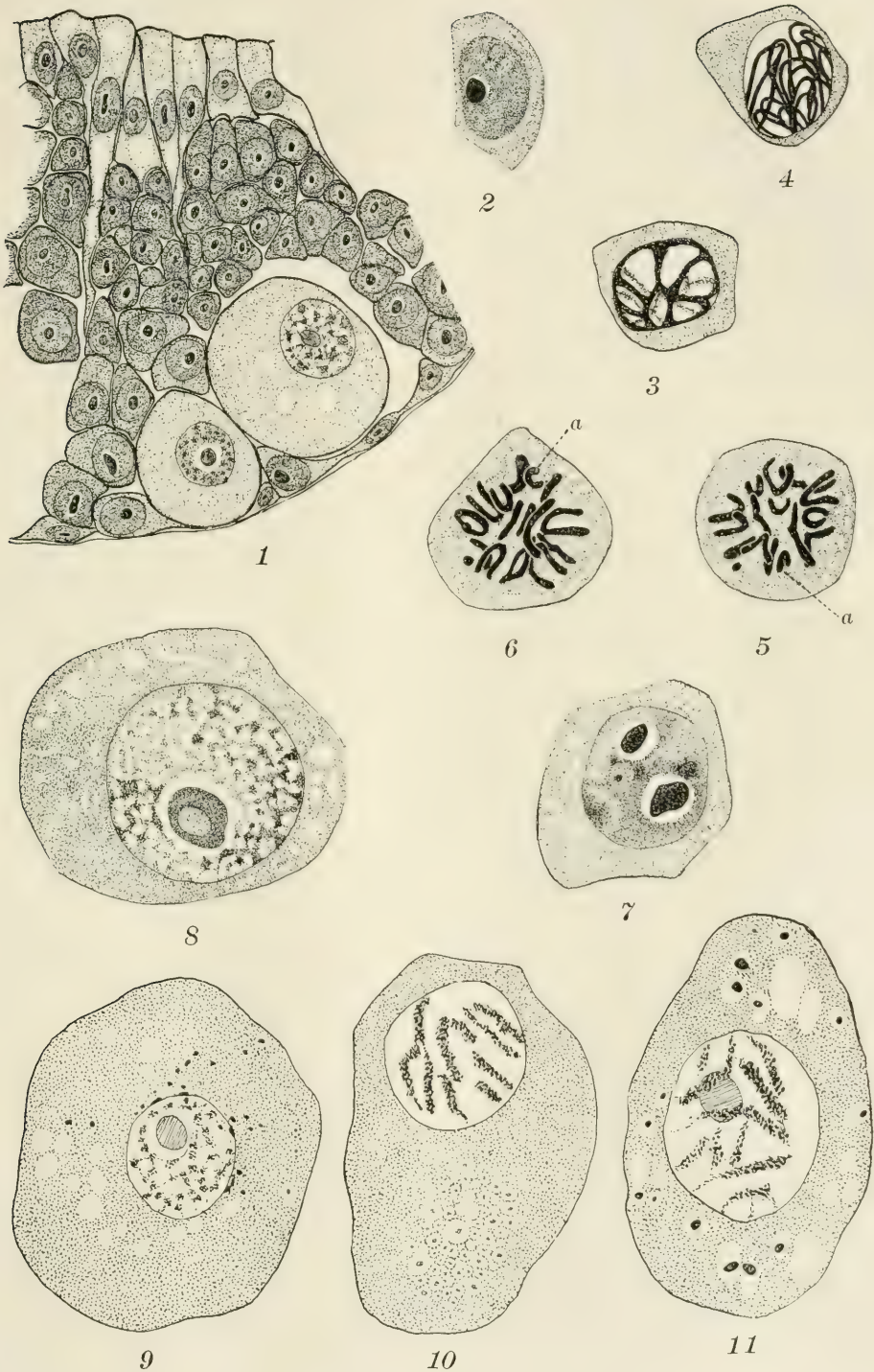


PLATE 2

EXPLANATION OF FIGURES

Aglantha digitalis

12 Egg nearly mature with germinal vesicle at periphery; chromatin condensing into chromosomes, nucleolus large and non-chromatic; chromatin grains in cytoplasm. $\times 1090$.

13 Prophase of first maturation with chromosomes free in cytoplasm; spindle not completely formed (4 chromosomes are present in adjacent section). Chromatin grains scattered in cytoplasm. $\times 1585$.

14 First maturation spindle with one chromosome lagging behind others in its division. The lightly shaded chromosome in the egg is placed from the adjacent section, other chromosomes of polar body in next section. $\times 1585$.

15 Telophase of first maturation division with the chromosomes splitting for the next division; each chromosome composed of granules. Some of chromosomes in adjacent sections. $\times 1585$.

16 Same as figure 15, all of the chromosomes (8) of the egg in vacuoles at inner pole of spindle. $\times 1275$.

17 Metaphase of second maturation; all of chromosomes of polar body present, one of egg chromosomes in adjacent section. $\times 2000$.

18 Egg nucleus and two polar bodies, the latter placed in position from the adjoining section. Some chromatin granules at side of egg nucleus. $\times 2000$.

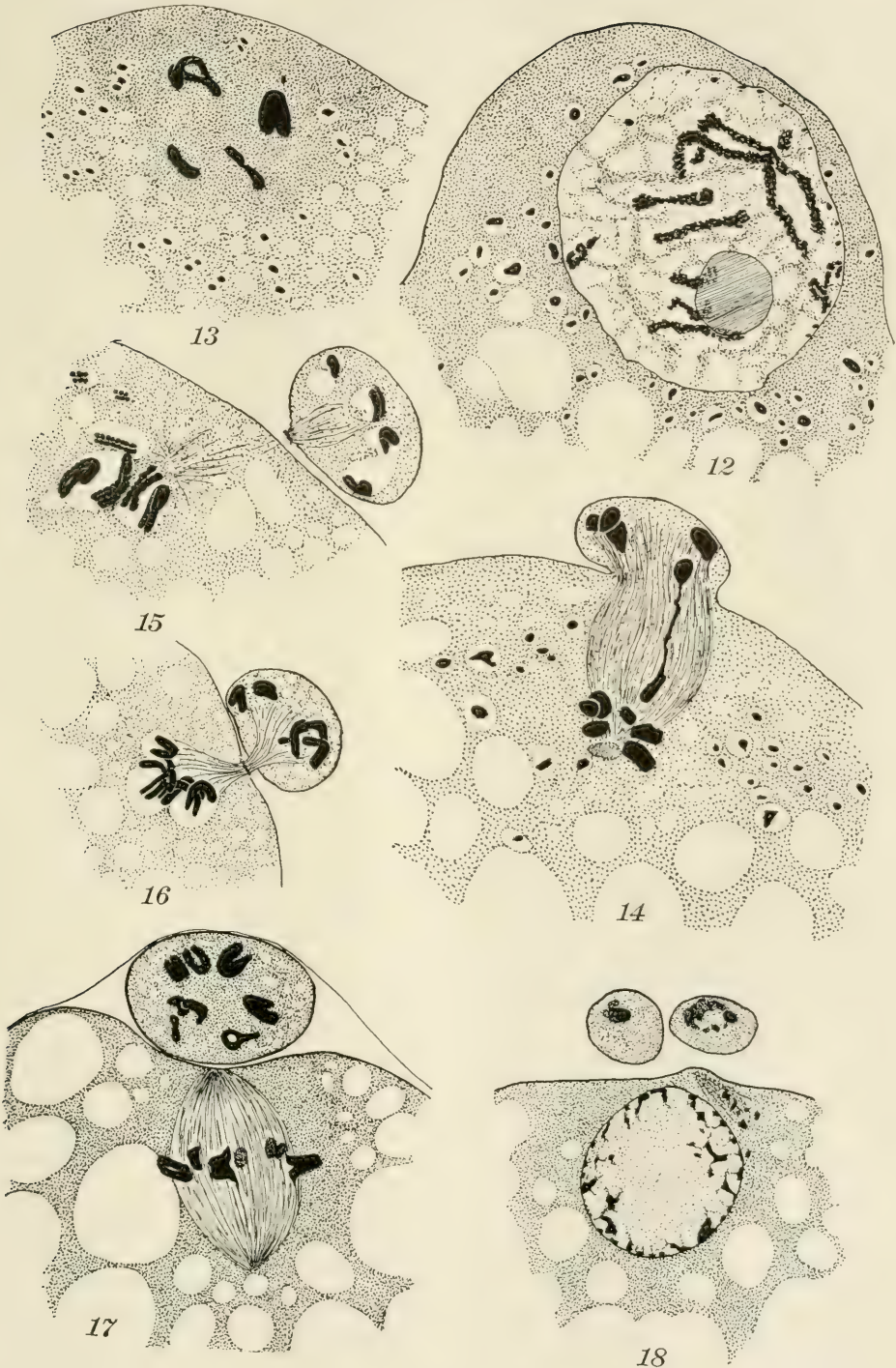


PLATE 3

EXPLANATION OF FIGURES

Aglantha digitalis

In all figures the chromosomes are represented as they appeared in the sections, all chromosomes were either present in a single section or if in two sections no single chromosome extended over more than one section. Figures 'a' and 'b' in each case represent the chromosomes of the same spindle which appeared in adjacent sections.

19 to 24 Side views of first maturation spindles in different phases. 19, 20, 24 $\times 1275$; 21 to 23 $\times 1585$.

25 to 27 Equatorial plates of first maturation mitoses. $\times 1275$.

28 Telophase of first maturation, side view of egg chromosomes grouped in vacuoles at pole of spindle. $\times 1275$.

29 Telophase of first maturation, polar view of egg chromosomes at end of spindle; the chromosomes are splitting in preparation for the next division. $\times 1585$.

30 to 34 Second maturation mitoses, equatorial plates. All $\times 1275$ except figure 32 which is $\times 2000$.

35 Telophase of second maturation mitosis, showing the group of chromosomes left in the egg. $\times 1275$.

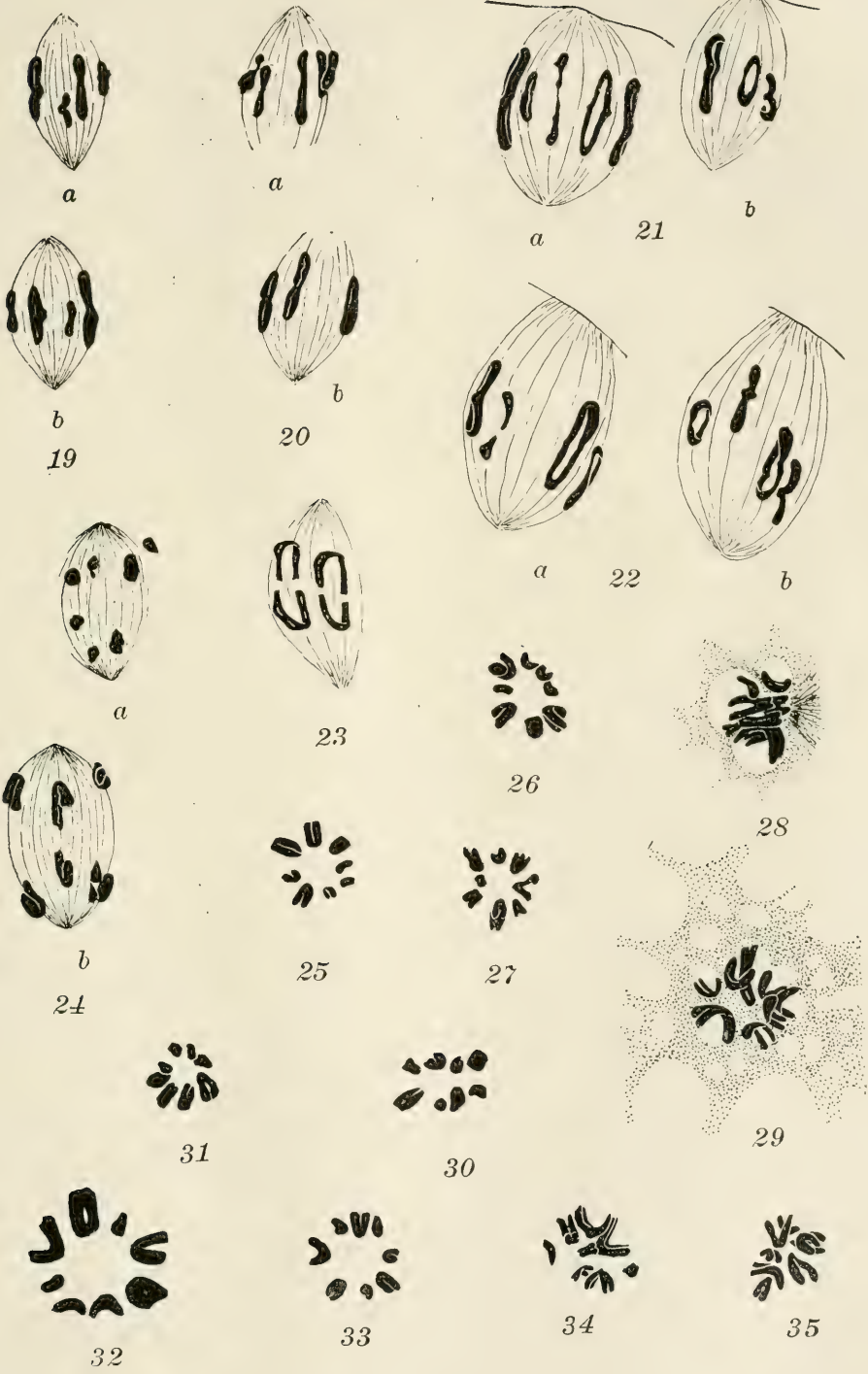


PLATE 4

EXPLANATION OF FIGURES

Hybocodon prolifer

36 The hydroid of *Hybocodon*, drawn from a preserved specimen. $\times 8$.

37 and 38 The medusae drawn from preserved specimens, in figure 38 the bell is considerably shrunken. The developing secondary medusae buds are shown, and in figure 38 the gonads on the stomach of the main medusa. $\times 8$.

39 Longitudinal section of entire medusa, slightly diagrammatic, compiled from several sections. Shows stomach with ovary and actinula, radial canals and marginal bulbs, the bulb on the right being the one from which secondary medusae arise. Portion of tentacle included with bulb on right. $\times 60$.

40 Longitudinal section through the radial canal and tentacle bulb. $\times 290$.



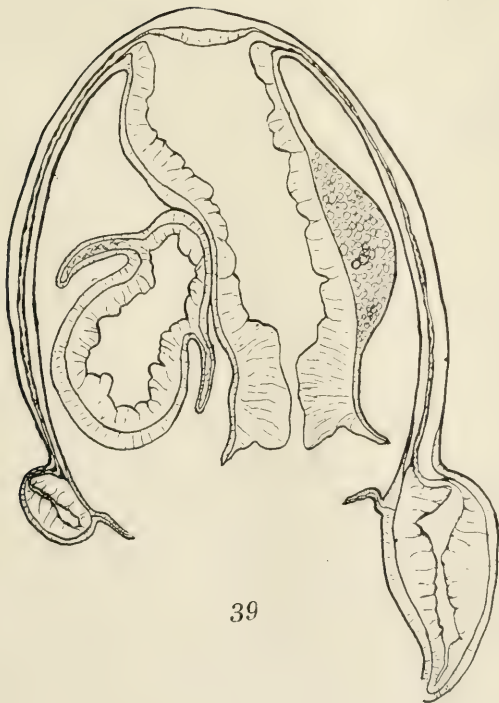
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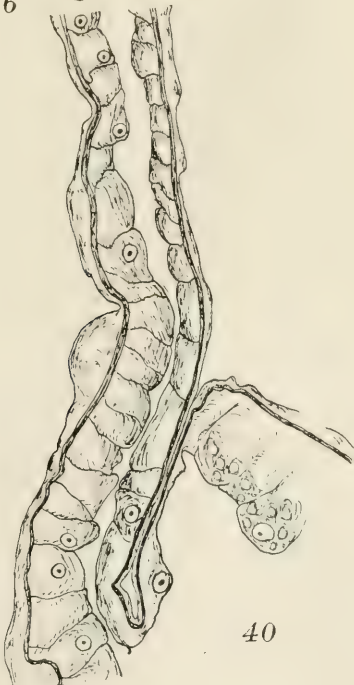
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PLATE 5

EXPLANATION OF FIGURES

Hybocodon prolifer

41 Cells of tentacle bulb of medusa to show the character of the ectoderm and entoderm; ectoderm to the left. $\times 615$.

42 Transverse section through radial canal of female medusa, near the aboral side of the bell. Entoderm and jelly shaded, ectoderm not shaded and cells not outlined. $\times 615$.

43 Longitudinal section of radial canal in the lower part of its course. Within the canal solid bodies, cellular in origin, in a state of disintegration. $\times 615$.

44 Transverse section of radial canal near its exit from the stomach. Within the canal is a cell of some sort. Only the entoderm shaded in figure. $\times 615$.

45 to 48 Various stages in the early development of secondary medusa buds. Figure 47 $\times 535$, others $\times 290$.

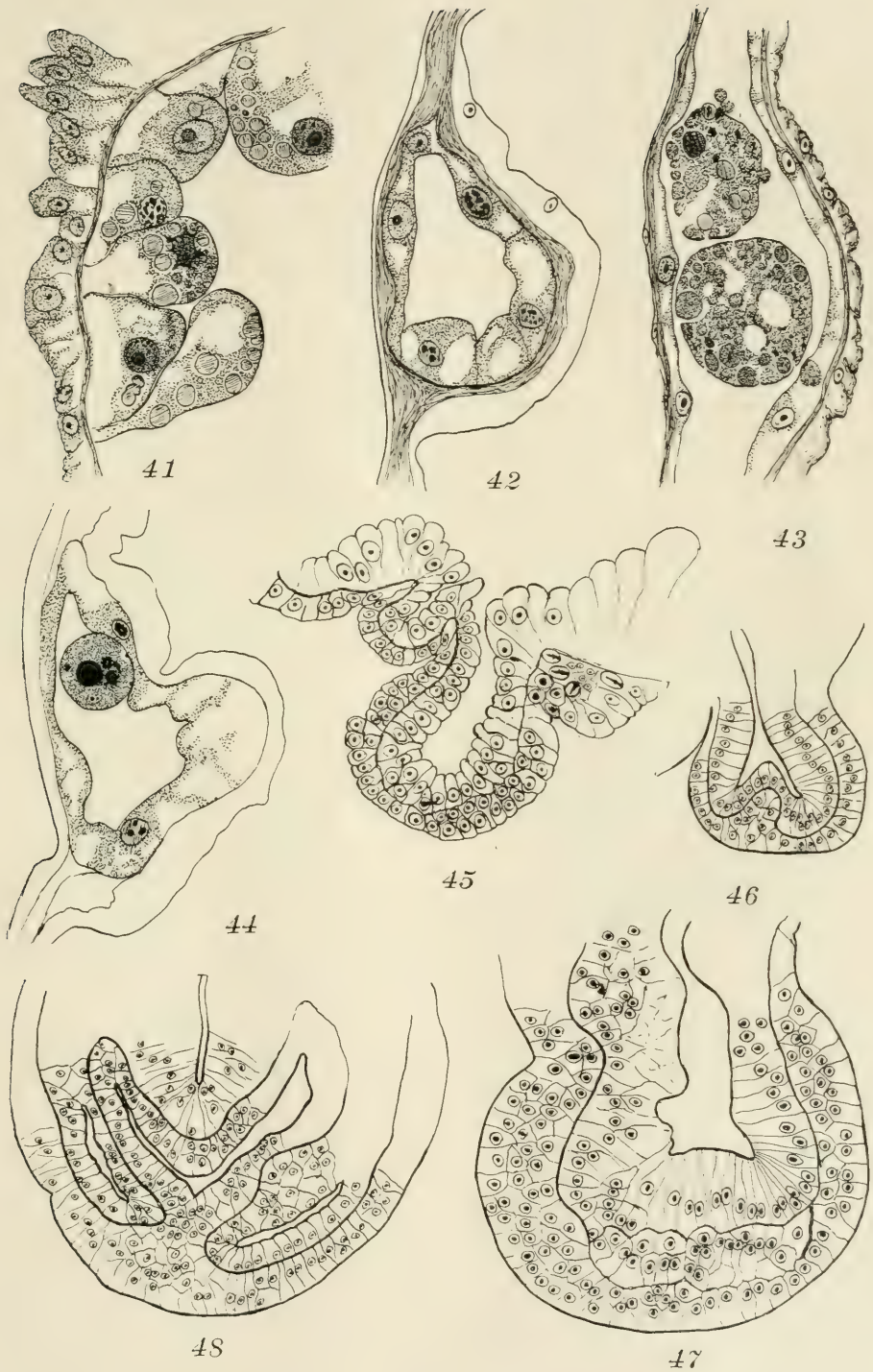


PLATE 6

EXPLANATION OF FIGURES

Hybocodon prolifer

49 Portion of a longitudinal section through stomach wall of developing secondary medusa, of a stage about like that of figure 48, ectoderm to the left. $\times 615$.

50 Part of a transverse section of stomach of developing secondary medusa, considerably older than figure 49, ectoderm uppermost in figure. $\times 615$.

51 Oocytes of a mature medusa, just ready to begin growth. Shows the character of cytoplasm, nucleolus, and nucleus; extra-nuclear chromatin in cytoplasm. $\times 1150$.

52 An oocyte from same ovary as figure 51; growth has begun. The cytoplasm and nucleus have undergone some changes. $\times 1150$.

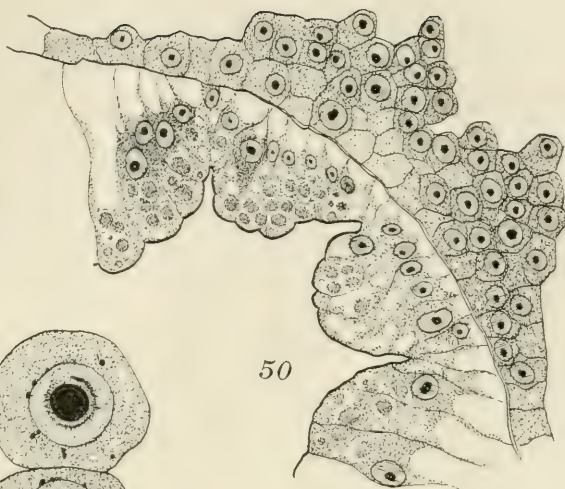
53 Growing egg of considerable size, but not full grown. Cytoplasm filled with degenerating nuclei of absorbed oocytes. Germinal vesicle shown. $\times 1150$.

54 Mature egg showing germinal vesicle; entoplasm of egg much vacuolated and containing chromatin grains, germinal vesicle contains small nucleolus and chromatin bodies. $\times 1150$.

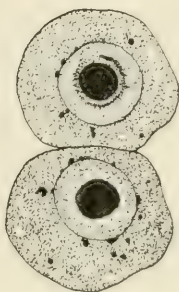
55 Reconstruction of stomach and growing eggs of one medusa; pseudopodia of eggs and the tiny germinal vesicles are shown; the oocytes which fill spaces between growing eggs not represented.



49



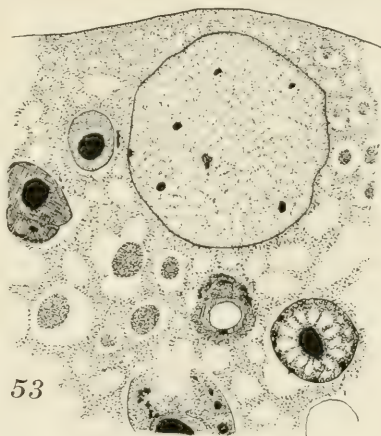
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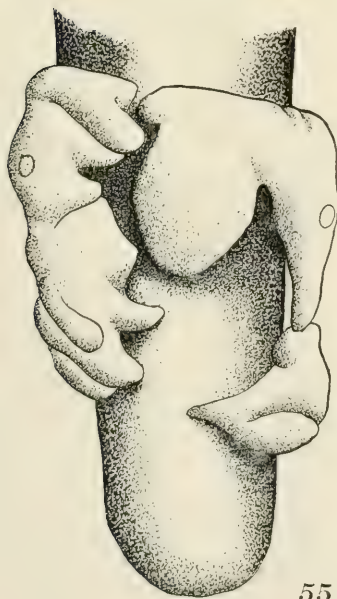
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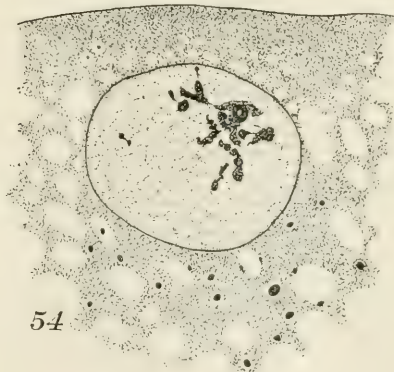
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CYTOPLASMIC INCLUSIONS IN MALE GERM CELLS OF THE FOWL TICK, ARGAS MINIATUS, AND HISTOGENESIS OF THE SPERMATOZOON¹

D. B. CASTEEL

ONE TEXT FIGURE AND EIGHT PLATES

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INTRODUCTION

This paper is more particularly concerned with the cytoplasmic elements found in the male germ cells of the chicken tick, *Argas miniatus*, with special reference to the occurrence and behavior of the mitochondria throughout the complete history of spermatogenesis, from spermatogonia to mature spermatozoa. As will be noted, the nuclear components of the cell are considered only in so far as they bear relations to extra-nuclear substances.

In view of the fact that the spermatozoa of many Acarines are of relatively large size and of markedly atypical form it is surprising to find that the literature dealing with them is but slight in amount. The more important studies upon the histogenesis of tick spermatozoa are those of Christophers ('06), Norden-

¹ Contribution from the Zoological Laboratory of the University of Texas, No. 129.

skilöld ('07), Bonnet ('07) and Samson ('09) as indicated in the latter portion of this paper. All of these investigators are concerned more particularly with the metamorphosis of the spermatozoon from the spermatid, and they give but brief and incomplete accounts of the preceding stages.

My attention was attracted to the growing spermatocytes and the spermatids of *Argas* by their relatively large size and the prominence of their cytoplasmic inclusions. This account represents the results of a work undertaken in the hope that a thorough study of these bodies throughout the history of the male germ cells might add to our knowledge of those problematical structures, the mitochondria, and that our data upon the spermiogenesis of the Acarines might be enlarged. For the sake of conciseness and to avoid needless repetition in the literature, reference is limited to papers which bear very directly upon the subject and no general review or discussion of cytoplasmic inclusions is attempted. Comprehensive reviews may be found in the works of Benda (1899, '03), Meves ('08, '10 et al.), Fauré-Fremiet ('10), Duesberg ('11), Hegner ('14), Cowdry ('12, '16) and others.

During the progress of my work the germ cells of several species of ticks have been examined for comparison. I am indebted to Mr. F. C. Bishopp of the United States Department of Agriculture for material of this nature sent to me from the Dallas Laboratory. I also wish to express my appreciation of the facilities afforded me during the summer of 1915 by Prof. F. R. Lillie at the Marine Biological Laboratory at Woods Hole. I am particularly indebted to Dr. E. V. Cowdry for certain vital stains and for helpful suggestions regarding technique and interpretation.

MATERIAL AND METHODS

The chicken-tick, fowl-tick or blue-bug, *Argas miniatus* (Koch), is a common parasite of fowls in the southwestern portion of the United States. It is synonymous with *Argas persicus* which is found in many parts of the world, in tropical and subtropical regions. The adult ticks are readily obtained from an infested

roost where they are often found in enormous numbers. During the day the adults and nymphs conceal themselves in cracks and crevices between the boards of the hen-house and from these hiding places they issue at night to prey upon the fowls. Only the seed ticks or newly hatched nymphs feed continuously upon the fowls, and this period lasts from but three to ten days. The life-history, habits and distribution of *Argas* are well described by Bishopp ('13).

All but the final stages in the development of the male germ cells of *Argas* may be obtained from the testes and sexual ducts of the male. The testes are two long tubes which lie loosely coiled within the body. Longitudinal sections through the testes reveal the germ cells in successive stages of development from the early spermatogonia onward, and such sections make possible the study of many stages from the same slide where they have all been subjected to the same fixing and staining technique. The final stages in development occur within the spermatophore sacs in the genital ducts of female and as the spermatozoa leave the spermatophores.

Both sections and smear preparations were employed and numerous observations were made upon the living cells both with and without vital dyes. Many fixing solutions were used, the most satisfactory being Flemming's stronger solution, and the fluids of Altmann, Benda, Meves and Bensley. Osmic acid (in 2 per cent solution and in the form of vapor) also gave good results. Bichromate-formalin was less satisfactory. The mixtures of Carnoy, Gilson and Bouin were used for purposes of comparison. Bensley's acid fuchsin with counter stain of methyl green, methyl blue, or toluidin blue gave the best differential results for mitochondria although the alizarin-crystal-violet method of Benda (or Duesberg's modification) is equally satisfactory in certain stages of development. Haidenhain's iron-haematoxylin was employed after all fixatives for comparison. As vital stains for mitochondria janus green (Hoechst) and its derivative diethylsafranin are most satisfactory. Certain special dyes, brilliant cresyl blue B extra, Sudan III, and others were found valuable for analysis. Prolonged study of the living

germ cells is rendered difficult by the rapidity with which they disintegrate after removal from the body of the tick. Even in the most suitable medium, a modification of Lock's solution, they remain normal for but a short time.

HISTORY OF THE SPERMATOCYTES AND SPERMATIDS

General account

As a basis for a review of the developmental processes which occur during the history of the germ cells of the tick it will be well to consider first the composition of a spermatocyte which is approaching the end of the growth period (fig. 9). The cell exhibits a distinct outer layer (*s.l.*) well demarcated from the underlying cytoplasm, composed of numerous excessively fine striae perpendicular to the surface. A tangential section through this layer or a surface view of the cell suggests the appearance of a faceted compound eye or of honeycomb. This region is referred to as the 'striated layer.' Between this area and the nuclear wall are three distinctly different types of inclusions within the otherwise homogeneous cytoplasm. The largest and most conspicuous of these structures (*v.b.*) are vesicular or sub-vesicular in form, some of them having the appearance of shortened cylinders, possibly open at the ends. In many respects these bodies fulfill the requirements of mitochondria, but, since they fail in certain particulars, they are here designated 'vesicular bodies.' At this period they take the counter stain with Bensley's acid-fuchsin-methyl-green or toluidum blue but may also show a tinge of red. The true mitochondria (*mi.*) appear as numerous minute granules well scattered throughout the cytoplasm. These granules often show a linear arrangement although they do not fuse to form distinct threads and they take the fuchsin stain. The third type of inclusion is represented by a number of small bodies (*e.b.*), much larger than the true mitochondria, which are scattered irregularly in the cytoplasm and which take the red of Bensley's stain. These structures vanish from the cytoplasm when the stage represented by figure 9 is reached, but may be seen in the five preceding figures. They are designated 'extra-

nuclear bodies.' A very distinct 'zwischenkörper' (not shown in figure 9) lies against the wall of the cell, the striated layer here being broken. No centrosomes are visible. The nucleus shows one (sometimes two or more) true nucleoli, and one chromatin nucleolus. At this stage the chromatin is diffuse in arrangement.

Turning now to earlier stages it will be noted that the true mitochondria are distinctly seen in spermatogonial cells before the beginning of the growth period (fig. 1). Indeed, these granules may be distinguished in stages much earlier than those represented. Following the last spermatogonial divisions, the mitochondria aggregate more densely in certain regions of the cell, more particularly against the nuclear wall (figs. 2 and 3) where they form one or more deeply staining caps. Thus far in development the cytoplasm appears entirely homogeneous except for the presence of mitochondria. Within the nucleus two nucleoli appear shortly after the end of the last spermatogonial division, and the chromatic material, which is at first diffuse, is more distinctly localized (fig. 3). Figure 4 marks the first appearance of extra-nuclear bodies and vesicular bodies. The former stain red with Bensely's acid fuchsin and are located both within and without the nucleus, many of them being in close contact with the nuclear wall. The latter are now in the form of short fibers, at first very difficult to resolve, but later increasing in bulk and becoming fusiform. They take the counter stain.

In figures 5, 6, 7 and 8, representing successive periods in the growth of the cell, the extra-nuclear bodies become more widely scattered in the cytoplasm. The vesicular bodies increase in number and are changing in form from the fusiform fibers of an earlier stage to the vesicular condition which they will retain until their disappearance. The mitochondria are more numerous and are evenly distributed throughout the cytoplasm. The genesis of the striated outer layer is seen in figure 7. At the end of the growth period (fig. 9) the striated layer has increased in thickness, the mitochondria maintain their uniform distribu-

tion, the extra-nuclear bodies have disappeared and the vesicular bodies are distinctly vesicular or cylindrical in form.

The details of the process of maturation will not be discussed in this paper since the behavior of the chromatic elements does not bear upon the history of the cytoplasmic inclusions. Immediately before the first maturation division the true nucleolus fragments and its elements disappear. The general arrangement of mitochondria and vesicular bodies during this cleavage is shown in figure 10, and figure 11 represents a spermatid immediately after the completion of maturation. In both maturation divisions mitochondria and vesicular bodies are apparently evenly distributed to the daughter cells.

Very shortly after maturation the spermatid exhibits distinct polarity. The nucleus migrates toward the surface of the cell and certain rearrangements occur among the cytoplasmic elements (fig. 12). The nucleus approaches a point on the surface which is about 30 degrees from the position of the *zwischenkörper* of the last cleavage. As the nucleus nears the striated layer the striae in its immediate neighborhood disappear. At the same time the mitochondria move in the opposite direction and begin the formation of an aggregate of mitochondrial granules (*a.m.*) at the opposite pole of the cell and somewhat removed from the striated layer. A movement of vesicular bodies toward the nuclear pole, more marked in later stages, begins at this time. Figures 12, 13 and 14 show successive stages in these cytoplasmic rearrangements, leading to the more marked differentiations shown in figures 15 and 16. Figure 15 shows the nucleus in contact with the cell wall which is here entirely without striations. The striated layer is now differentiated into two regions, one around the equatorial region of the cell which exhibits no further modification, and a cap-like area at the pole opposite the nucleus where the striations are shorter. The mitochondrial condensation has resulted in the formation of a massive 'mitochondrial ring' (*m.r.*, fig. 16) which lies slightly below the equator of the cell but which in later stages moves toward the nucleus. Nearly all of the mitochondria of the cell are concerned in the formation of this ring and many of the

smaller granules fuse together to form large irregular masses. At no time in their history do the mitochondria take their characteristic stains more readily than now, and in the living cell this ring is a most striking object. Figure 15 also shows the manner in which the vesicular bodies approach the nucleus, although, as a matter of fact, they should scarcely be spoken of as vesicles at this time since they are apparently breaking up. Lying mostly between these structures and the mitochondrial ring are now seen certain other substances (*o.d.*) which appear as homogeneous globules in the living cytoplasm and which, as will later appear, are to be considered as oil droplets.

Between the stage represented by figure 15 and that in figure 19 striking changes occur in the general form of the cell, leading toward the formation of the spermatozoon. The vesicular bodies entirely disappear and the oil droplets increase in number. Little change occurs in the constitution of the mitochondrial ring except a further condensation of its component granules and the migration of the ring as a whole toward the nuclear pole of the cell. The external form of the cell, however, is modified by the invagination of that region which lies immediately opposite the nuclear pole (*i.e.*), while at the same time the striated layer becomes transformed into a mass of cilia-like threads (*c.p.*) which project into the invagination cavity. These threads remain short in the region formerly marked by the cap of shorter striations while in the former equatorial region the threads are longer than the original striations (fig. 18). The oil droplets show no change.

From this period until the outer tube of the spermatozoon develops the changes which occur are shown in figures 19, 20, 21, 22, and 23. The mitochondrial ring loses its distinct form and the large granules of which it is composed break up to form smaller granules (figs. 22 and 23). The nucleus moves to one side of the cap of cytoplasm. The cell wall immediately beneath the shorter cilia-like threads thickens and these threads elongate to form a little tuft which projects downward into the invagination cavity (figs. 22 and 23). The opening into the cavity becomes progressively smaller.

The further history of the metamorphosis of the spermatid into the spermatozoon will be deferred until the latter portion of this paper.

The true mitochondria

The granular bodies identified as mitochondria are undoubtedly substances of this nature since they fulfill the more generally accepted criteria, although their responses to various methods of technique are not always the same throughout the entire history of the germ cells. The general statement may be made that, up to the time the granules aggregate to form the mitochondrial ring, they are relatively inconspicuous features of the cytoplasm, being difficult to resolve in living cells and staining less sharply with those dyes which are accepted as specific, although there is never any doubt of their presence. Evidently some change occurs in their chemical organization at about the time the mitochondrial ring forms. In stages earlier than this the granules are completely dissolved in fixatives containing a large per cent of acetic acid, but the ring itself, although partly dissolved, is usually not entirely obliterated under such treatment. Somewhat similar variations in the resistance of mitochondria to acetic acid have been noted by Regaud ('08) and by others. With Benda's stain, following Meves' solution, the granules of earlier stages show the violet color but faintly, while the ring takes this stain intensely. In living cells janus green and diethylsafranin stain the ring much more intensely than they do the scattered granules of preceding stages. However, osmic acid darkens the granules and the ring with about equal intensity and the same is true of iron-haematoxylin following osmic fixation.

The question of the manner in which the mitochondria of *Argas* increase in number is extremely difficult to answer. As has already been noted, such an increase probably occurs to some extent throughout the growth of the germ cells, and probably at a rapid rate just before and during the process of maturation. While conclusive evidence is not at hand for absolute statements, my material warrants the belief that the addition

of new granules is not by formation *de novo*, but results from the growth and division of preëxisting granules. In stages represented by figures 8, 9, 10 and 11 the mitochondria are of different sizes and the larger granules show irregularities in shape which might be interpreted as indicating constrictions which would lead to division. Moreover, the linear arrangement of the granules might be interpreted as representing series of granules which had been produced by division of one or more original granules. However, the granules are so minute at this time that one is not justified in making dogmatic statements. Most of the mitochondria of the cell are concerned in the aggregation which forms the ring. Some, however, remain scattered in the cytoplasm. The disintegration of the ring, shown in figures 21, 22 and 23, results from the splitting up of mitochondrial masses, although the resulting bodies are not as small as the granules which originally formed the ring.

Extra-nuclear bodies

The various accounts and discussions which have appeared in recent years relative to the nature and, indeed, the reality of substances in the form of globules, granules, exudations, etc., supposedly emitted from the nucleus of various animal cells, give particular interest to the presence of similar structures in the spermatocytes of *Argas*. It is not my purpose to discuss the literature of this subject here, but merely to state the facts in this particular case with a few brief comparative references. The structures which are designated extra-nuclear bodies (*e.b.*) appear soon after the stage represented by figure 3 and they vanish shortly before maturation. Figure 3 may be considered as representing a 'pre-emission' stage. At this time an acid-staining nucleolus, a basic-staining nucleolus and the chromosomes are the only structure within the nucleus which react to dyes, while within the cytoplasm the mitochondria and the *zwischenkörper* take definite stains. With the continued growth of the cell small bodies, globular rather than granular, appear within the nucleus and they are also seen within the cytoplasm,

either applied to the nuclear wall or in its immediate neighborhood, and they are later more widely scattered in the cytoplasm. A glance at figures 4 to 8 will show the positions occupied by these bodies. Before the time of maturation they entirely disappear. From the morphological standpoint the evidence is very conclusive that these bodies arise in the nucleus, pass to its periphery, reach the nuclear membrane, and in some form and in some manner pass through the wall, later to become free in the cytoplasm. The passage of these substances through the wall has not been observed, but in several cases the nuclear wall is bent outward in front of the body and somewhat modified in texture, but these conditions may represent artefacts.

Regarding the nature of these bodies the following suggestions are offered. They do not represent the 'extranuclear chromatin' of Schaxel ('11) and others, for their staining reactions do not suggest chromatin, nor are they similar to the pseudochromatin granules of Beckwith ('14) either in point of origin or in microchemical reactions. In many particulars these bodies recall the nuclear globules of Beckwith, for they bear like positional relationships, react similarly to Benda's stain and, after a brief existence, they disappear from the cytoplasm. They are not mitochondria and appear to bear no definite relation to the mitochondria, for acid fixatives such as Carnoy's fluid have no effect upon them, while dissolving all of the mitochondria in the cell. Moreover, the mitochondria are already present in the cell when these bodies appear. The extra-nuclear bodies are present in the cytoplasm when the first rudiments of the vesicular bodies are becoming visible, but they are not transformed into these latter structures, for the vesicles are also dissolved by strong acetic solutions; nor is there any morphological evidence of such a transformation. That the presence of extra-nuclear bodies in the cytoplasm gives the chemical stimulus necessary for the formation of the vesicular bodies, is possible but scarcely probable.

The cellular structure with which the extra-nuclear bodies appear to be most closely related in microchemical reactions is the true nucleolus or plasmosome. After Benda's stain the

extra-nuclear bodies correspond in color to the plasmosome and do not stain like the chromatin, and with Bensley's acid-fuchsin-methyl-green (or other counter stain) they and the nucleolus take the brilliant red of the acid dye. One is led to the conclusion that these extra-nuclear bodies represent products of nuclear activity similar to those substances found in the plasmosome and that they are of little further significance in the economy of the cell, being thrown out into the cytoplasm where they are later dissolved and absorbed after the manner in which the plasmosome itself disintegrates at the time of maturation.

Vesicular bodies and oil drops

As has been noted, the vesicular bodies are first seen during the early growth stages, having the form of elongated, fusiform fibers which appear among the mitochondria, both where these granules are thickly aggregated and where less concentrated. There is no evidence that the fibers arise directly from mitochondria as described by Sokolow ('13) for structures of somewhat similar form in the spermatocytes of the scorpion, but they appear to arise *de novo* in the cytoplasm. There is some evidence that the fibers may multiply by transverse fission, although this is doubtful. The metamorphosis of a fiber into a vesicle is accomplished by the thickening of the fiber in the middle, followed by the appearance of a cavity in the thickened portion, which gradually increases in extent until a distinct vesicle is formed (figs. 5, 6 and 7). When the stage represented by figure 8 is reached, all of the fibers have been transformed into vesicles and some of the vesicles are cylindrical in shape with thin-walled ends. Apparently these bodies increase in number during the remaining period of growth but no evidence can be presented as to the exact method of multiplication.

Following maturation, the vesicular bodies take part in the polarization of the spermatids by separating from the true mitochondria and moving toward the nucleus. Figures 12 to 15 illustrate these rearrangements and they also show the beginnings of certain degenerative processes which lead to the disinte-

gration of the vesicles (figs. 17 and 18). These changes are marked by the breaking of the walls of the vesicles or cylinders (so that sections of these bodies are now sickle-shaped or thread-like) and by their dissolution. As the vesicles degenerate the cytoplasm in which they lie shows the presence of rounded homogeneous globules or droplets (*o.d.*). The point of origin and the position of these globules strongly suggest a causal relationship between their appearance and the disintegration of the vesicular bodies. However, it can not be urged that any single globule is formed directly from a particular vesicle, for the globules are larger than the vesicles. The substance comprising the globules is of an oily or fatty nature, if microchemical tests may be relied upon, for the globules become blue when the cells containing them are placed in a modified Lock's solution to which is added brilliant cresyl blue in proportions of 1: 50,000, while other elements of the cell remain unstained.

How shall the vesicular bodies be classified? Apparently they are closely allied to true mitochondria, although they fail to conform in all particulars to the criteria employed in identifying typical mitochondria. They agree with mitochondria in that (1) they are dissolved in acetic acid; (2) they are not unlike mitochondria in form; (3) they show certain typical mitochondrial reactions to dyes. With Bensley's stain the vesicles take the fuchsin but slightly, and the same is true of the violet of Benda's stain. In the living cell the vesicles are but lightly stained with janus green, but this is also true of the mitochondria before they aggregate to form the mitochondrial ring, nor are the true mitochondrial granules of the spermatogonia and spermatocytes at all well differentiated with Benda's stain. As has already been noted, the responses of mitochondria to microchemical tests apparently differ at different periods in the life of the cell which contains them. The granules which are undoubtedly mitochondria pass from a state of relative indifference to certain supposedly specific stains (as crystal violet and janus green) to a condition in which these stains are taken with avidity. The vesicular bodies may be thought of as mitochondrial in nature but differing from the granules in this particular,

that they disintegrate before reaching a stage in chemical composition which corresponds to that attained by the true mitochondria at the time they form the ring. The association of fat globules with the vesicular bodies at the time of their disintegration also suggests an identity with typical mitochondria, if the results and conclusions of Dubreuil ('11, '13) are accepted, for he derives fat droplets very directly from mitochondria, and a similar relation is suggested by Fauré-Fremiet ('10) and others for certain types of mitochondria. On the other hand, the work of the Lewises ('15) throws doubt upon such a relationship, although the possible influence of mitochondria in fat formation is not denied.

Finally the possibility should be considered that the vesicular bodies may represent intra-cellular parasites. It is claimed by Hindle ('11) that the organism (*Spirochaeta gallinarum*) which produces spirochaetosis in fowls, passes through a stage in its life history in which the spirochaete breaks up to form many small, rounded bodies which he speaks of as 'coccoid bodies.' These bodies are found in various tissues of the tick but more particularly in the cells of the Malpighian tubules and in the ovary. Marchoux and Couvy ('13) deny that this relationship exists between the so-called 'coccoid bodies' and the spirochaete and they claim that these bodies are normally present in the tissue cells of species of ticks which do not carry protozoan organisms. They are unable to decide upon the nature of these structures, although, among other possibilities, they suggest a relationship to mitochondria. A thorough study of cells from the Malpighian tubules, salivary glands, crop and intestine of *Argas* leads me to the conclusion that none of the cytoplasmic inclusions found in the cells of these organs is to be identified as at all similar in structure or behavior to the vesicular bodies of the germ cells. In the spermatocytes and spermatids of other species of ticks which I have examined (*Dermacentor variabilis*, *Ornithodoros turricata*, *Rhipicephalus sanguineus*) are found inclusions entirely homologous with the vesicular bodies of *Argas*. The manner in which the vesicles appear at a particular stage in the history of the germ cells, and the way in which they pass

through a series of definite modifications at all times parallel with certain developmental stages of the cell, argues strongly against a parasitic relation. Moreover, although *Spirochaeta gallinarum* is a common blood parasite of the fowl and its tick in many parts of the world, it has never, to my knowledge, been observed in the blood of chickens or in ticks living in the United States. The evidence seems conclusive that the vesicular bodies are component parts of the germ cells and are not of external origin.

FORMATION OF THE SPERMATOOA

The preliminary steps in the metamorphosis of the spermatid have already been indicated. The stage shown in figure 23 is produced by the invagination of that side of the cell which lies opposite the nucleus (fig. 19), and this invagination marks an important phase in the series of transformations which eventually produce the highly specialized spermatozoon of the tick. These processes begin with the extension of the invagination cavity, and soon this cavity with its surrounding walls is converted into a hollow tube (*o.t.*) which rapidly extends outward from the nuclear region of the cell until it reaches the relatively enormous length shown in figure 27. This tubular outgrowth is designated the 'outer tube.' In the early stages of its development the cilia-like remains of the striated layer of the spermatid project into the cavity of the tube, but these structures degenerate later and form a gelatinous mass which partly fills the tube. The shorter filaments which are grouped to form a tuft at the bottom of the tube retain their individuality somewhat longer.

While the outer tube is developing, certain changes are occurring at the nuclear end of the elongating cell. The mitochondria break up into yet finer granules which collect around the proximal end of the tube (fig. 24). The cytoplasm opposite the base of the tube now flows outward and somewhat to one side, at first in the form of a blunt projection which becomes more extended (fig. 25) and finally takes the form of a distinct finger-like process (*f.p.*) as shown in figures 26, 27 and 28. During the period represented by figures 23 to 27 the nucleus also undergoes exten-

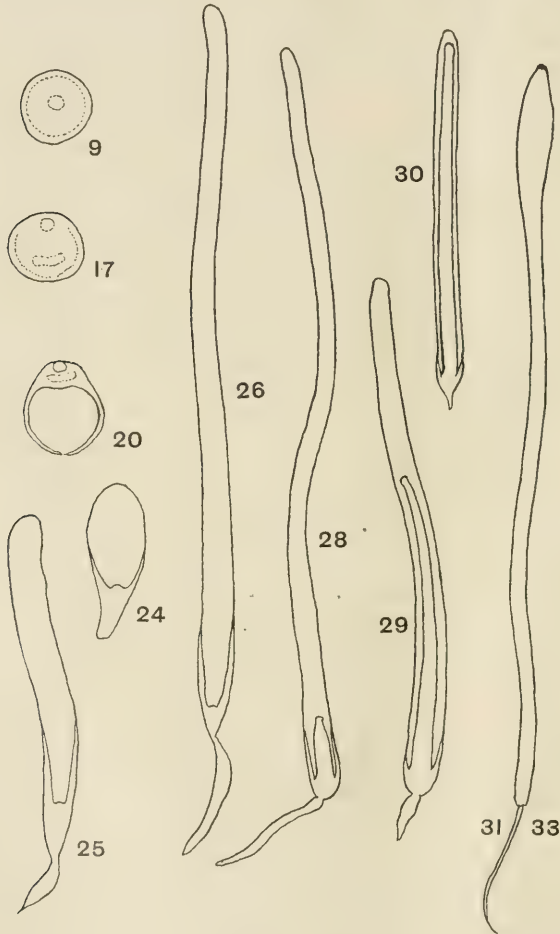
sive alteration, becoming pear-shaped in form and later more elongated. The chromatin, which heretofore has been diffuse, aggregates into threads which stain intensely with nuclear dyes. The nucleus now orients itself in relation to the finger-like process in such a manner that the more pointed end projects into the basal portion of this process.

During the course of its formation the outer tube is bent upon itself and coiled around its basal region although the arrangement of the loops is not always similar to that shown in figure 27. At this stage the developing spermatozoa are packed closely together, and only in teased preparations and in smears can their individuality be distinguished. As the tube grows outward it is surrounded by a gelatinous envelop (*g.e.*) which increases in amount as the tube develops and apparently serves to hold the coils of the tube together. This enveloping gelatinous mass persists until the spermatozoa are near the completion of their metamorphosis.

Figures 27 and 28 picture the genesis of a structure designated the 'inner tube' which arises within the proximal end of the outer tube. As will be recalled, this region of the original invagination is marked by a cluster of cilia-like processes which extend into the cavity. Even in the stage represented by figure 21 the surface of the cell is here thickened. In later stages this thickening becomes more extensive and finally develops into a deeply staining ring-like elevation which projects from the bottom of the outer tube and which bears a circlet of delicate threads (figs. 24, 25 and 26).

At about the time the outer tube reaches a condition of maximum extension this ring begins to project into its cavity (fig. 27). This is the beginning of the formation of the inner tube (*i.t.*) which continues to advance within the outer, as shown in figures 28 and 29, until both tubes reach approximately the same length (fig. 30). As a matter of fact the inner tube grows at the expense of the outer, for during its formation the length of the developing spermatozoon is reduced about one-half, as will be seen from a comparison of figures 27 and 30, and as is shown in text-figure 1. As the head of the inner tube advances,

the substance which forms the walls of the proximal half of the outer tube is drawn into the basal region of the inner tube. From the very beginning of its formation the inner tube contains mitochondria, and as its growth progresses it draws into itself all of the mitochondria of the cell (figs. 27, 28, 29, and 30).



Text fig. 1 Outline drawings of illustrative stages in the formation of the spermatozoon, showing its relative form and size at different periods of development. All stages here represented are from camera lucida drawings of living material at the same magnification. The numbers refer to approximately similar stages shown in the plates.

The mitochondria are densely collected at the outer or distal end of the inner tube and when the tube is fully formed all of these granules lie in its distal third. Between the stage shown in figure 27 and that of figure 28 the cilia-like processes upon the tip of the inner tube vanish, and in their place is a clear hemispherical vesicle which may possibly represent the substance formerly seen in these processes.

During the growth of the inner tube the nucleus migrates extensively. As shown in figure 28 it still lies at the bases of the two tubes, but shortly afterwards it moves around to the outer surface of the outer tube and begins to travel along this tube toward the opposite end. The outer tube is at this time covered with a gelatinous sheath through which the nucleus moves. By the time the inner tube has reached its full extension the nucleus lies opposite its distal end, and upon the outer surface of the outer tube (fig. 30). While these rearrangements are occurring, the finger-like process progressively shortens (figs. 29-30), and some of the substance of which it was composed is apparently absorbed into the basal ends of the two tubes.

In the condition shown in figure 30 the spermatozoa leave the male tick and their development is completed within the genital ducts of the female, where they are at first packed together in sac-like spermatophores, and later lie freely in the oviduct. Figure 31 represents a spermatozoon from a spermatophore sac. By comparison with figure 30 it will be noted that the older spermatozoon is now in the form of a single tube with the mitochondria at one end and the nucleus at the other, and that its total length is about twice that of the spermatozoon shown in figure 30. See also text-figure 1. In accomplishing this metamorphosis the mitochondria-filled end of the inner tube pushes through the distal end of the outer tube (at *a*, fig. 30) and continues its advance, while the outer tube slips backward along the outer surface of the inner tube until at last outer and inner tubes are merged into one continuous structure. As a result of this process the outer tube is turned completely inside out, and the end which was formerly around the mitochondria-bearing end of the inner tube now lies at the opposite end of the spermatozoon.

(fig. 31). The nucleus retains contact with the original outer surface of the outer tube and is carried to the opposite end of the spermatozoon, where it now lies within the tube. The remnant of the finger-form process and the gelatinous envelop of the original outer tube are also carried within the tube as it slips backwards and turns inside out. Within the nuclear end of the completed tube, as represented in figure 31, is a long, somewhat coiled, flagellum-like structure (*f*) which extends downward in the tube from the mass of protoplasm which surrounds the nucleus.

The final steps in metamorphosis occur just before the spermatozoon leaves the spermatophore sac and immediately thereafter as it lies within the oviduct. At this time the flagellum pushes past the nucleus and outward through the open end of the tube (fig. 32) and soon is completely protruded (fig. 33). The nucleus also slips out of the tube and lies imbedded in the base of the flagellum. The opposite or mitochondria-bearing end of the sperm tube now changes somewhat in form. It becomes flattened and ridged as shown in figure 34 and a circlet of short processes appears around the vesicle which crowns the tip-end of the tube.

The extremely bizarre form of the spermatozoon of the tick makes difficult the task of homologizing its parts with those of other spermatozoa. In the case of *Argas* about the only landmark which one can use is the nucleus, for no centrosomes are visible at any stage of spermatogenesis, and the highly complex processes of metamorphosis obliterate or mask the characteristic features of typical spermatozoa. Using the final position of the nucleus as a guide, one is led to the conclusion that the morphological head of the spermatozoon is that end of the completed tube in which the nucleus lies. One would naturally expect that this would be the forward end of the moving spermatozoon but such is not the case. The living spermatozoon of *Argas* does not exhibit locomotion until it is completing its development in the spermatophore sac in the oviduct of the female, at which time it moves with the mitochondria-bearing end in advance. This observation is in harmony with the conclusions

of Samson ('09) for the spermatozoa of *Ixodes* and *Ornithodoros*. Samson also holds the view here expressed, that the nucleus marks the morphological head of the mature sperm tube, basing her conclusions upon the identification of the nucleus by its staining reactions and upon her studies of fertilization. After many fruitless attempts she succeeded in observing the process of fertilization in *Ixodes* and she states that it is the nuclear end which first penetrates the egg. I have endeavored in vain to secure similar data for *Argas*.

If it be granted that the nucleus marks the morphological head of the tick spermatozoon one might infer that the protoplasmic process (flagellum), which pushes outward from the nuclear end of the tube at the time the spermatozoon reaches maturity, is homologous with the acrosome of the other spermatozoa. The origin of this structure is somewhat uncertain since the cytoplasm of which it is composed takes no specific dye, but it may not be too much to infer that it is formed largely of the substance of the conspicuous finger-shaped process which is so prominent a feature of the earlier stages shown in figures 25 to 29. This process does not migrate along the primary tube with the nucleus and, although it becomes reduced in size and relatively inconspicuous by the time the spermatozoon is transferred to the female, it yet persists and must later be drawn into the nuclear end of the completed tube when the outer tube has slipped completely back over the inner.

The exact manner in which the spermatozoon of the fowl-tick accomplishes locomotion is difficult to determine. As the mitochondria-bearing end advances, its vesicular tip contracts and expands in a peculiar manner and the processes surrounding the vesicle are also in motion. The mitochondria within the tube lie near the surface and the granules are arranged in linear series, giving this portion of the tube a longitudinally striated appearance. One gains the impression from an examination of living spermatozoa that the mitochondria represent contractile elements which are responsible for the movements which this end of the spermatozoon exhibits. A relation between mitochondria and motor organs was long ago suggested by

Benda ('99), and more recently Duesberg ('10) has advanced the idea that the myofibrils of striated muscle are of mitochondrial origin. As has been noted, mitochondria are found only in the end of the sperm-tube which shows active movement.

Samson's ('09) accounts of the metamorphosis of the spermatozoa of *Ixodes ricinus* and *Ornithodoros moubata* are in many respects parallel to that of *Argas* as here related. Starting with a very brief description of the spermatid of *Ornithodoros* (illustrated by two figures) she follows the formation of both inner and outer tubes and notes the manner in which the nucleus migrates along the outer surface of the outer tube. She also notes, but does not represent in her figures, the presence of a 'finger-förmigen Plasmafortsatz,' the fate of which is left in doubt. The later stages in metamorphosis are taken from the history of the spermatozoon of *Ixodes*. The mature spermatozoon is figured and described, and the statement is made that in the processes of its formation the head of the inner tube pushes its way through the outer, but the manner in which this evagination leads to the formation of a single tube is not definitely indicated. An apical organ is described and its origin from material within the completed tube is suggested together with the relation of this body to the nucleus. The presence of a flagellum-like structure ('geissel') within the nuclear end of the tube is indicated, but no stage is figured in which this flagellum projects from the tube as it does in *Argas*, although supposedly mature spermatozoa of both *Ixodes* and *Ornithodoros* are shown. Samson reviews the work of Christophers, Bonnet and Nordenskiöld, pointing out some of their errors, to which further reference will not be made in this paper.

SUMMARY AND CONCLUSION

In the spermatocytes of *Argas miniatus* toward the end of the growth period three distinct types of cytoplasmic inclusions may be recognized. These are designated true mitochondria, extra-nuclear bodies and vesicular bodies. The outer surface of the cytoplasm is differentiated to form a honeycomb-like

layer marked by striations which run perpendicular to the surface.

The extra-nuclear bodies appear early in the growth period. They are first seen within the nucleus, later pass through the nuclear wall, and finally become widely scattered in the cytoplasm, where they are dissolved and absorbed before the time of maturation. Their behavior and staining reactions suggest that these bodies are similar in nature to the plasmosome or true nucleolus.

The vesicular bodies are formed *de novo* in the cytoplasm at about the time the extra-nuclear bodies appear, but no apparent relationship exists between the two. When first seen they appear as fusiform fibers but as they increase in number the fibers open out to form vesicles (*v.b.*, fig. 5). These bodies are evenly distributed in the cytoplasm until after the formation of the spermatids, when they collect near the nucleus at one side of the cell. Here they gradually disintegrate and, as they are breaking down and disappearing (fig. 15), droplets of a fatty nature (*o.d.*) are deposited in the cytoplasm which surrounds them. The vesicular bodies are considered as mitochondrial in nature for they fulfill several, although not all, of the tests employed in determining mitochondria.

The true mitochondria are present in the youngest spermatogonia examined, and they persist, with certain modifications of arrangement and form, throughout the entire history of the developing spermatozoon. In the young spermatids (fig. 13, *a.m.*) mitochondria begin to collect at the side of the cell opposite the nucleus, where many of the granules fuse together and form a massive ring (figs. 15 and 16, *m.r.*). Later, when the mitochondrial ring breaks up (fig. 23) the granular mitochondria are not widely scattered but are carried in a mass to a particular region of the forming spermatozoon.

The transformation of the spermatid into the spermatozoon is initiated by (1) the migration of the nucleus to one side of the cell, (2) the formation of the mitochondrial ring (3) the movement of the vesicular bodies toward the nucleus and their later disintegration, and (4) by the disappearance of the outer striated

layer from the surface of the cell near which the nucleus lies. See figures 12 and 15. Following these rearrangements, the surface of the cell opposite the nuclear pole is indented and the bounding walls of the invagination cavity thus produced (fig. 19) grow outward (fig. 23) to form a long tube which is designated the 'outer tube' of the developing spermatozoon (fig. 24 and following). Into this tube is carried the material which formed the outer striated layer of the spermatocytes and spermatids. The striations now take the form of cilia-like processes (although they are not mobile), which later dissolve and form a gelatinous mass within the tube. However, a tuft of these threads at the base of the tube persists for a longer period (figs. 24, 25, 26, 27). At the opposite or nuclear pole of the cell a finger-form process (*f.p.*) develops and the nucleus migrates toward the base of this process, at the same time changing in form (figs. 24 and 26).

The next step in metamorphosis is the formation of the 'inner tube' which develops within the outer tube (figs. 27, 28, 29 and 30). The inner tube grows at the expense of the outer, for when both are of nearly equal length, the length of the entire spermatozoon is reduced by one-half. The nucleus migrates through the gelatinous layer along the outer side of the outer tube (fig. 29) and eventually lies opposite the distal ends of both tubes (fig. 30). All of the mitochondria of the cell are drawn into the distal end of the inner tube. The finger-form process is reduced in size.

While the spermatozoon is in the spermatophore sac within the genital ducts of the female, the distal end of the outer tube forces its way out of the inner tube (at *a*, fig. 30), and the outer tube slips backward along the inner. With the completion of this evagination process, the two tubes form one continuous sperm-tube, one-half of which represents the inner tube and the other half the original outer tube turned inside out (fig. 31). The mitochondria now lie at one end of the spermatozoon, within the original inner tube, while the nucleus, which has retained contact with the original distal end of the outer tube and has been carried backward with it, now lies within the end

of this tube and at the opposite end of the continuous sperm-tube. A flagellum-like process (*f*), which is probably derived from the finger-form process, lies within the nuclear end of the tube.

Metamorphosis is completed by the protrusion of the flagellum from the nuclear end of the spermatozoon (fig. 33) and by the formation of a circlet of mobile processes at the opposite or mitochondria-bearing end of the sperm. In locomotion the end of the spermatozoon which bears the mitochondria goes first and it would appear that the contractile elements which make locomotion possible are of mitochondrial origin.

The end of the spermatozoon which bears the nucleus is interpreted as the morphological head, and the flagellum (which is not mobile) probably represents the acrosome of more typical spermatozoa.

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PLATES

EXPLANATION OF PLATES

All figures are drawn to the same scale except figure 29, which was drawn to one-half the scale of the others. The drawings were all made at table level with a Leitz camera lucida and with the use of Leitz ocular 6 and Leitz 2 mm. apochromat N. A. 1.32 objective, draw tube set at 152 mm. With one-third reduction in reproduction the magnification is thus 926 diameters (except figure 29). At all stages in the development of the spermatozoon size variations may be noted. In most cases an average-sized representative of a particular stage has been selected, but the figures will show some exceptions to this practice. Only those cytoplasmic constituents which are essential to the discussion are represented in the drawings; otherwise distinctions could not be expressed satisfactorily with line drawings. Unless otherwise indicated the drawings are made from sections 2 to 5 in thickness.

PLATE 1

EXPLANATION OF FIGURES

- 1 Spermatogonial cell in division. The small dots in the cytoplasm in this and the figures which follow represent mitochondria.
- 2 Two cells at the beginning of the growth period. The nuclear cap is formed of mitochondria.
- 3 Three growing cells from a testicular cyst.
- 4 Early growth stage showing genesis of extra-nuclear bodies (*e.b.*) and vesicular bodies (*v.b.*).
- 5, 6, 7 and 8 Successive stages in the growth period. Figure 7 shows the first evidence of the striated layer (*s.l.*).

ABBREVIATIONS

<i>a.m.</i> , aggregating mitochondria	<i>m.r.</i> , mitochondrial ring
<i>c.p.</i> , cilia-like processes	<i>n.</i> , nucleus
<i>e.b.</i> , extranuclear bodies	<i>o.d.</i> , oil droplet
<i>f.</i> , flagellum	<i>o.t.</i> , outer tube
<i>f.p.</i> , finger-form process	<i>pl.</i> , plasmosome
<i>g.e.</i> , gelatinous envelop	<i>s.l.</i> , striated layer
<i>i.c.</i> , invagination cavity	<i>v.b.</i> , vesicular bodies
<i>i.t.</i> , inner tube	<i>z.</i> , zwischenkörper
<i>mi.</i> , true mitochondria	

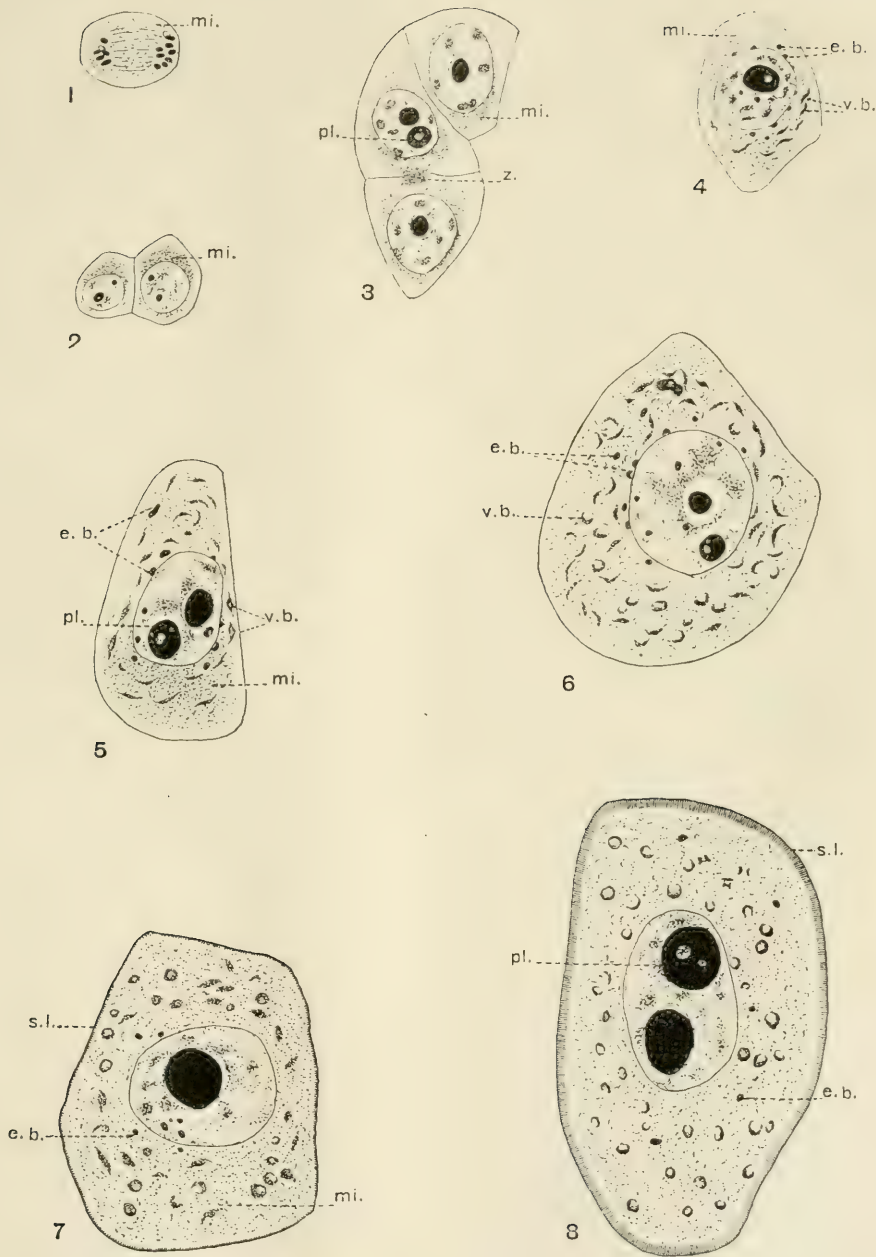


PLATE 2

EXPLANATION OF FIGURES

- 9 Primary spermatocyte just before division.
- 10 First maturation division.
- 11 Spermatid immediately after completion of second maturation division.
- 12 Spermatid showing beginning of polarization of the cell as expressed by the aggregating mitochondria (*a.m.*) and by the manner in which the striated layer is vanishing from the region of the cell which the nucleus is approaching.
- 13 and 14 Spermatids in which the process of polarization is continued.

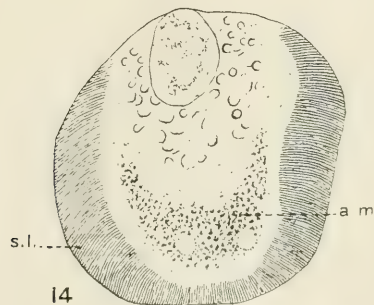
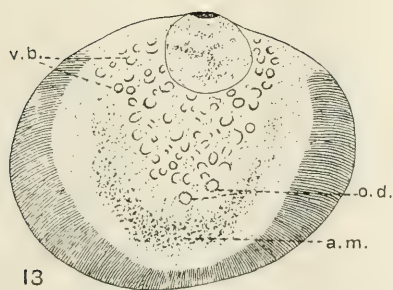
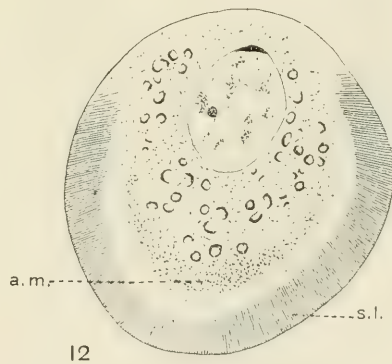
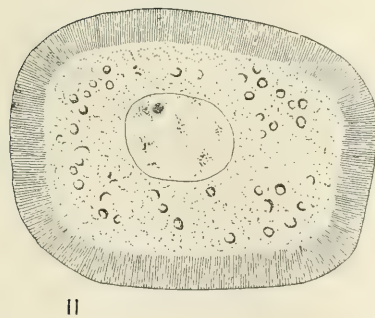
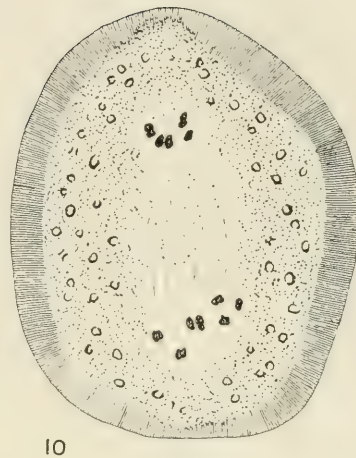
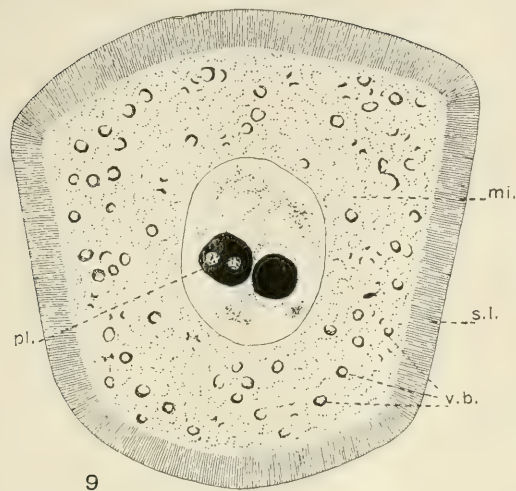


PLATE 3

EXPLANATION OF FIGURES

15 Spermatid exhibiting differentiation of the striated layer into two regions, disintegration of vesicular bodies, deposition of oil droplets (*o.d.*) and the completion of the mitochondrial ring (*m.r.*).

16 Section of the cell perpendicular to that of figure 15, showing the form of the mitochondrial ring.

17 and 18 Two stages preceding the invagination of the spermatid. The vesicular bodies vanish, additional oil droplets appear and in the striated layer the formation of cilia-like processes (*c.p.*) begins.

19 and 20 Two stages in the development of the invagination cavity (*i.c.*).

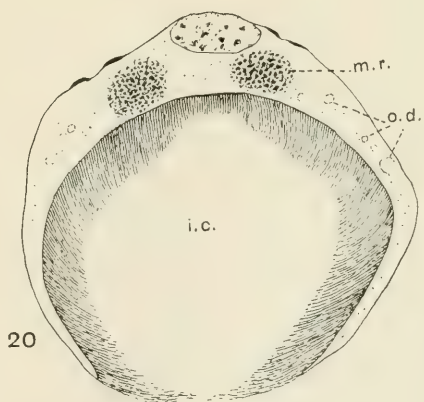
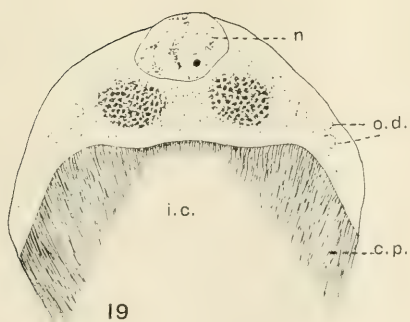
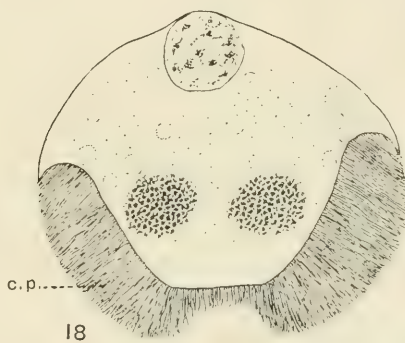
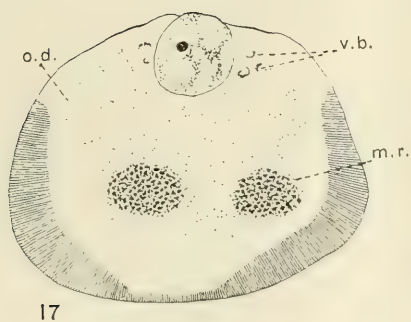
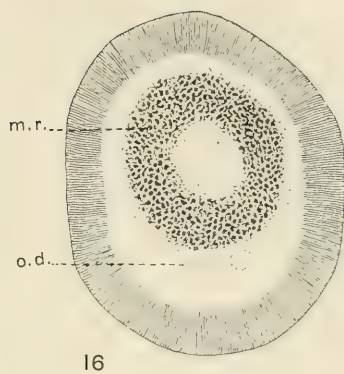
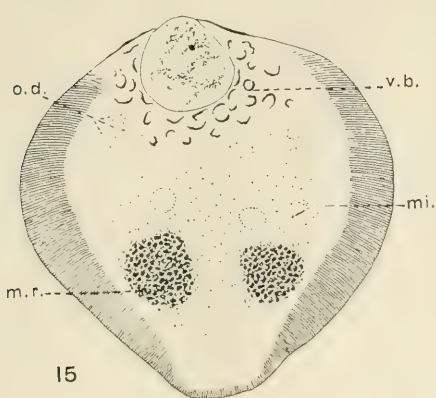


PLATE 4

EXPLANATION OF FIGURES

21 and 22 Two stages representing the enlargement of the invagination cavity and the breaking up of the mitochondrial ring.

23 The beginning of the elongation of the invagination cavity.

24 The invagination cavity is elongating to form the outer tube (*o.t.*) of the spermatozoon, the nucleus is changing in shape and the finger-form process (*f. p.*) is appearing.

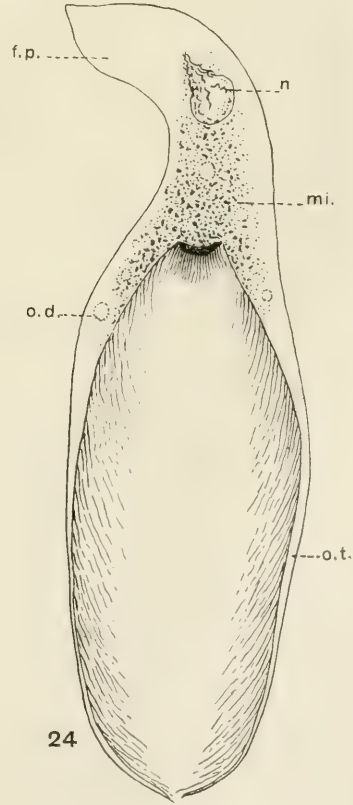
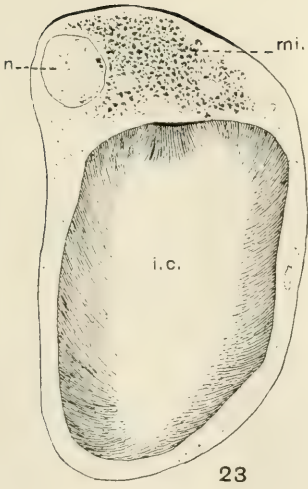
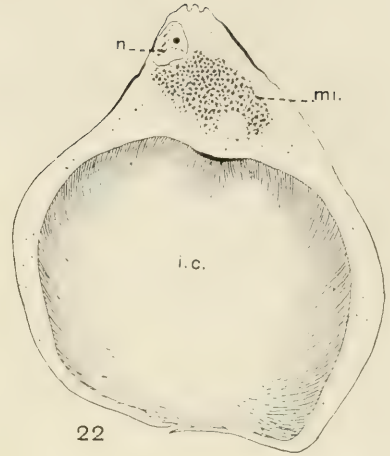
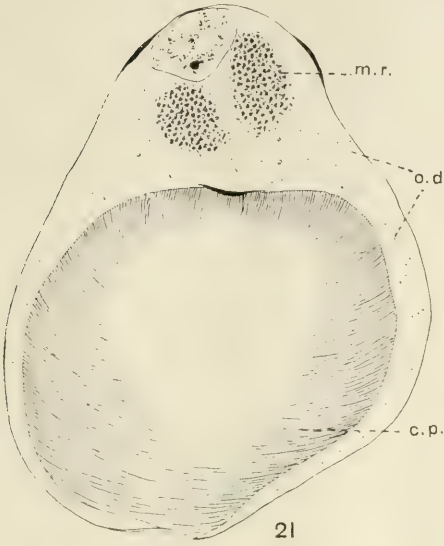


PLATE 5

EXPLANATION OF FIGURES

25 Proximal end of a developing spermatozoon. The finger-form process is taking shape.

26 Proximal end of a developing spermatozoon. The finger-form process has reached its maximum size. The cilia-like fibers within the inner tube have largely disintegrated, except those at the bottom of the tube.

27 Entire young spermatozoon from smear preparation, showing the long, coiled outer tube somewhat surrounded by the gelatinous envelop (*g.e.*) and the beginning of the formation of the inner tube (*i.t.*) with the mitochondria flowing into it.

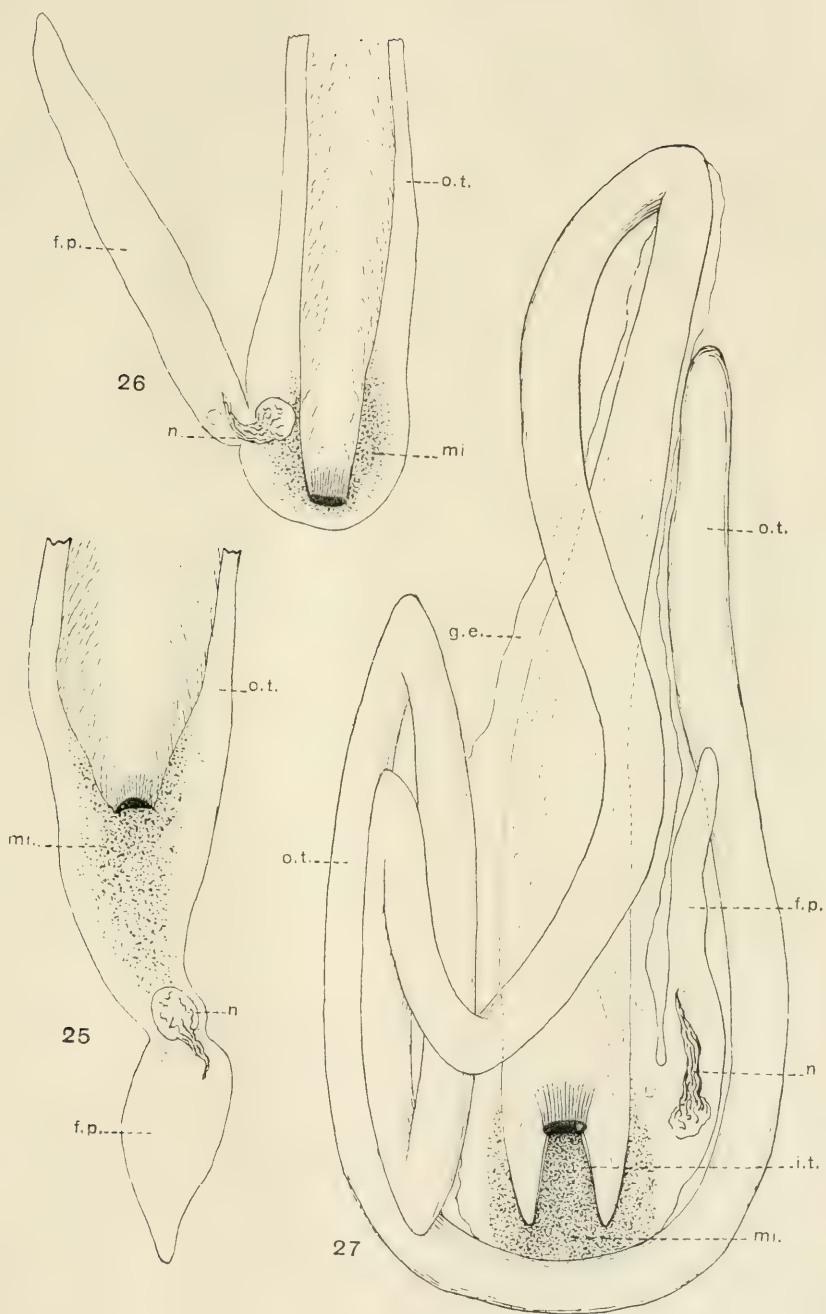


PLATE 6

EXPLANATION OF FIGURES

28 See plate 7.

29 Young spermatozoon from smear preparation, drawn to one-half scale of the other drawings. The head of the inner tube has advanced half way down the outer tube. The elongated nucleus is migrating through the gelatinous envelop toward the distal end of the outer tube. The finger-form process has diminished in size.

30 Spermatozoon from smear preparation in the condition in which it is transferred in the spermatophore from the male to the female tick. Both the nucleus and the mitochondria-bearing end of the inner tube lie near the distal end of the outer tube. The inner tube will later break through the outer at the point *a*.

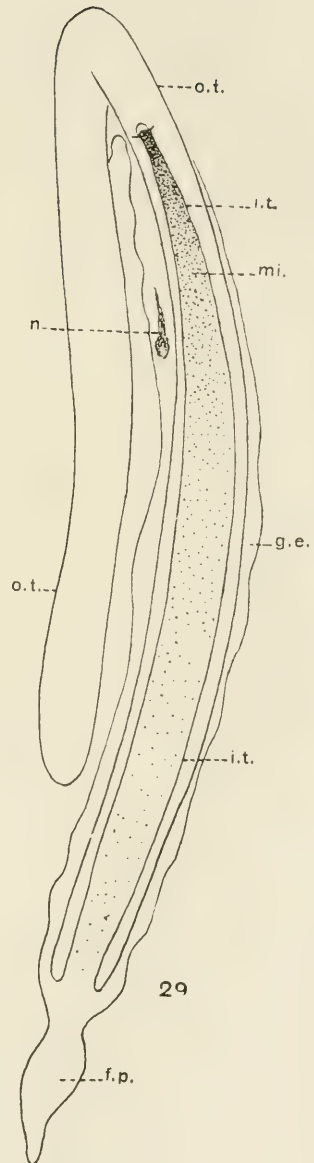
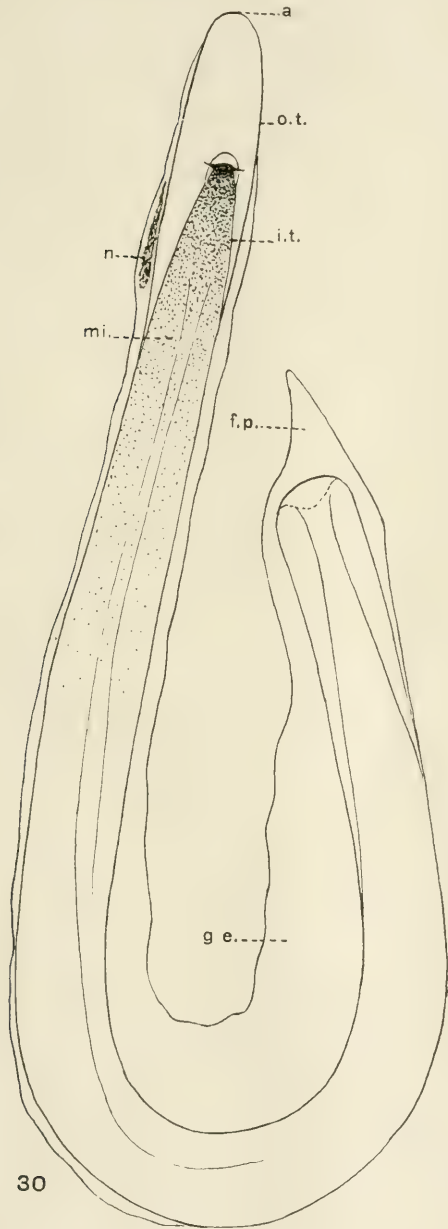


PLATE 7

EXPLANATION OF FIGURES

28 Proximal end of young spermatozoon showing further growth of the inner tube. The cilia-like fibers at its apex have given place to a clear vesicle.

31 Spermatozoon from smear preparation, showing the transformation which occurs while it is within the spermatophore sac. The complete evagination of the inner tube from within the outer results in the formation of one continuous tube. The nucleus and the mitochondria now lie at opposite ends of this tube and the nucleus, together with much of the gelatinous envelop and the finger-form process is now within the tube. The flagellum (*f.*) hangs down within the tube. The dotted lines represent the length of that portion of the spermatozoon which is omitted from the drawing.

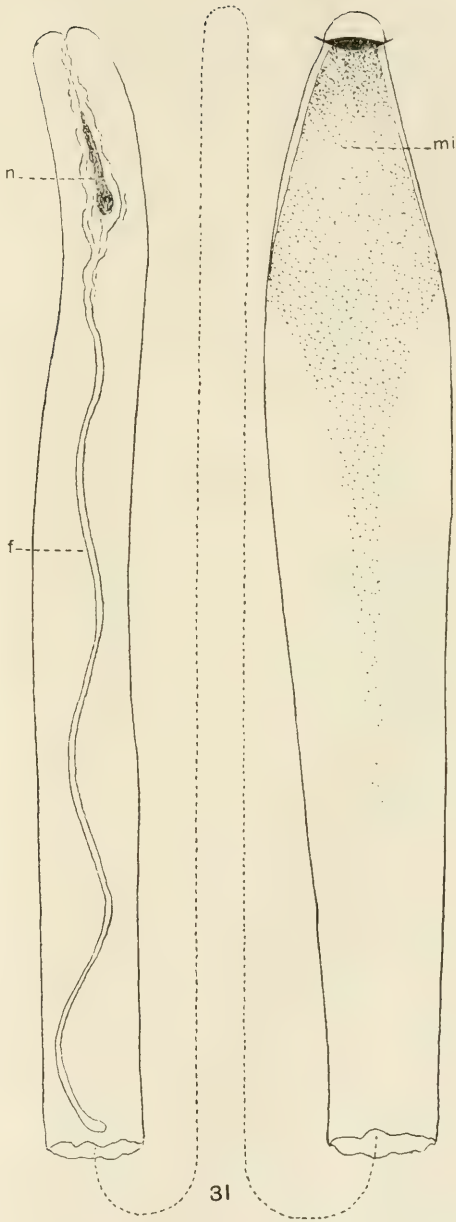
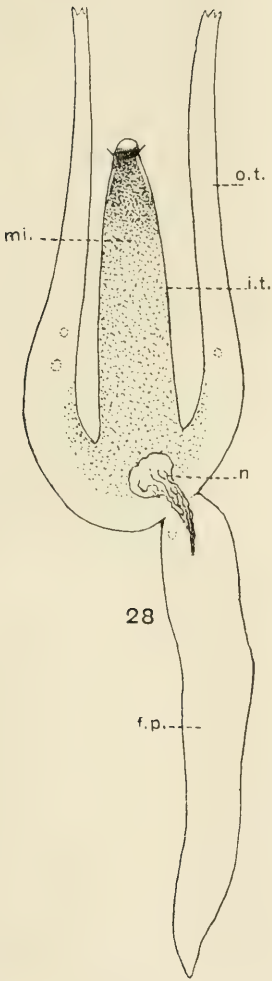


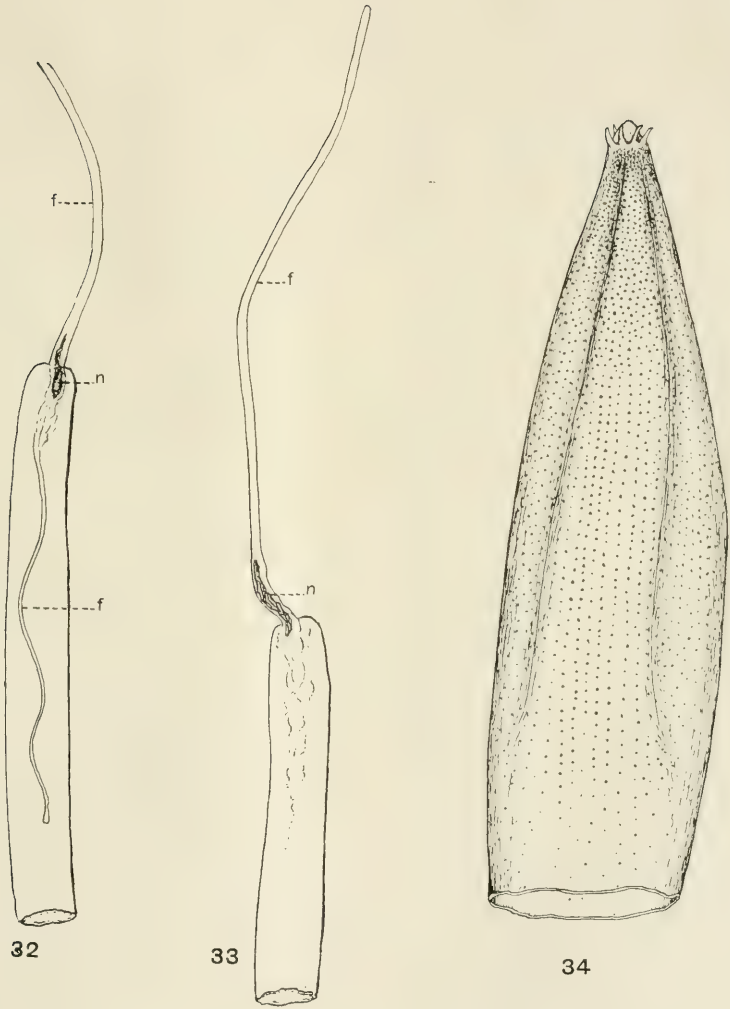
PLATE 8

EXPLANATION OF FIGURES

32 Nuclear end of living spermatozoon in which the flagellum is beginning to protrude from the tube.

33 Nuclear end of living spermatozoon with flagellum fully extended.

34 Mitochondria-bearing end of a living spermatozoon in the same stage of development as that in figure 33. The vesicle at the tip is surrounded by a circle of short processes. The tube is ridged and somewhat flattened.



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